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SUPPLEMENT 341

Studies on the Mechanisms of the Electrophonic Effect

BY

GORDON FLOTTORP, -

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Studies on the Mechanisms of the Electrophonic Effect

BY

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Preface

his monography on electrophonic hearing is the ... lt of more than 25 years work My interest in the theory of hearing was initiated by my teacher and ief professor doctor J Holtsmark His friendship ith doctor Georg von Bekesy resulted in Bekesy visiting Oslo University during his stay in Stockholm The group of young acousticians working at the Physical Institute A at that time was greatly nspired and encouraged by Bekesy s advises and lectures

The third printing of «Hearing Its Psychology and Physiology» by S S Stevens and H Davis (1948) was a basic textbook at our Institute Beside my work on difference limen for frequency and intensity I started experimenting with electrophonic hearing More or less by change I discovered the fricative effect Persuaded by professor Holtsmark I applied for a governmental scolarship in order to continue the study at the Psycho Acoustic Laboratory at Harvard and got it Professor S S Stevens as the director of this famous laboratory and G von Bekesy as its senior research fellow welcomed me in October 1950 to Harvard

My stay at Harvard was prolonged and I continued my work on electrophonic hearing as a research assistant

My first paper on electrophonic hearing «Effect of different types of electrodes in electrophonic hearing» appeared in Journal of Acoustical Society of America March 1953

It was my intention that this publication should be supervened by a work about the various physical mechanisms involved in electrophonic hearing

My appointment in 1952 as an audiophysicist at the just founded Institute of Audiology at the University of Oslo brought about new activities in the field of audiology The study of electrophonic hearing had to be slowed down Professor O Opheim the head of the Institute and of the Ear nose throat department of Rikshospitalet encouraged however the work and helped providing the special human material wanted for the further study

A paper on «The electrophonic effect in ears lacking ear drum» was presented at the meeting of the Scandinavian Acoustical Society in Stockholm 1958

In October 1964 I was asked by the Committee on Hearing Bioacoustics and Biomechanics («CHABA») – being and international correspondent of CHABA – to evaluate a research report «Electro-Stimulation Techniques of Hearing»

This resulted in a welcome opportunity to increase my research on electrophonic hearing An account of my evaluation of the research report together with results of my own experiments was sent to CHABA in March 1965

Thanks to a six months leave from routine duties during winter 1971/72 I could consentrate on the ambitious goal to solve the transducing mechanism in the Brenner method of electrophonic hearing At that time the Institute had moved to a new building with excellent facilities for research and the staff was increased Professor doctor Peter Berdal the successor to professor Opheim greatly favoured my efforts to solve remaining problems and complete the planned monography

The renewed interest in electrical stimulation of hearing by means of implanted electrodes has encouraged the finishing of the work so long under way

In presenting the monography I want to accknowledge quite a few persons without the inspiration and help of which the work should never have been done The two most important persons in this connection professor doctor S S Stevens and professor doctor G von Bekésy are no longer alive I consider it one of my most important privileges to have worked under their guidance and to have been accepted as their friend and co-worker benefiting from their unmatched knowledge about the ear and hearing and from their brilliancy of experimental endeavour

Bekésy s interest in my work on the fricative effect on leather together with his subtle humour is

reflected in a poem placed on my desk November 1 1951 (At that time he worked on cochlear microphones and we discussed the peculiar similarity between wet leather and the inner ear with respect to transducer action)

Leder Vers

und doch ist es verlockend

Druckt man es periodisch
so entsteht ein Wechselstrom
doch ist dies nicht komisch
denn es ist genau wie beim Mikrophon

Doch geht ein Strom durchs Leder
so entsteht ein feiner Ton
und sofort weiss ein jeder
dies ist ein neues Sympton

Hier bin ich nun in den Vereinigten Staten
und vor mir liegt das Leder
doch glaube ich man muss lange warten
bis die Losung fliesst in meine Feder

The staff members of the Psycho Acoustic Laboratory in 1950-52 have «suffered» as my ...jects and provided valid data as excellent ...vers The same is to be said about the staff of The Institute of Audiology and the Ear nose throat department of Rikshospitalet during various periods from 1953 to 1975 I am grateful to my three Norwegian bosses Holtsmark Opheim and Berdal for encouragement and provision of possibilities to carry the work through

In solving the many technical problems encountered I have received outstanding help from Rufus L Grason and Ralph Gerbrands at the Psycho-Acoustic Laboratory J Sandstad Physical Institute – Oslo University A Enger O Vik M Szalay A Bjelde S Solberg and A Sundby at The Institute of Audiology – Oslo University Of the many ENT physicians at the ENT department of Rikshospitalet helping with the experiments on patients Aa Rypdal and J G Hall should be specially thanked for also having served as subjects A warm thank is extended to the many patients who so kindly without complaints have served as subjects in experiments being many times rather boring

In preparing the manuscript valuable suggestions and corrections have been given by dr P Andersen dr E Jellum dr Sv Quist Hanssen mr G Nesheim and members of the staff at the Physical Institute and of The Institute of Audiology The typing and several re typing of the manuscript have been carried out by our friendly secretaries E Finnanger E Bagger and M Lund

My wife and three children must be honoured for their patience and indulgence rarely seeing me home in time for dinner and for family activities

In thankful reverence to the founder of The Institute of Audiology for his continual economical support of the research at the Institute this book is dedicated to shipowner dr h c Anders Jahre

Acknowledgments

Acknowledgment is made to The Journal of the Acoustical Society of America American Institute of Physics New York New York for permission to use the figures in my publication in J Acoust Soc Am 25 1953 236 245 and to MacDonald and Jane s Publishers London England and the authors Bockris and Reddy for permission to use Fig 2 6? in Modern Electrochemistry Volume 1 in the present monography

1. Introduction

The term «Electrophonic effect»

The notion of an «electrophonic phenomenon» was formed by S S Stevens (1937) as a result of experimental work on hearing by electrical stimulation At that time the findings of Wever and Bray (1930) had been examined more thoroughly and the separation between cochlear microphonics and action potentials in the eighth nerve had been established (Saul and Davis 1932) It was stated that the ear behaves as an electromechanical transducer i e when the auditory mechanism is stimulated by a sound wave it generates an identical electric wave known as the cochlear potential or aural microphonic Stevens (1937) considered the effect to be reversible in accordance with other transducer mechanisms His findings pointed in the same direction as an alternating current passed through the head an auditory sensation resulted He called this phenomenon *electrophonic hearing* or the *electrophonic effect*

In a later publication the phenomenon was more precisely defined «The production of an auditory sensation by the passage of an electric current through the head constitues the phenomenon of electrophonic perception» (Jones Stevens and Lurie 1940) Research has shown that under this conception of the term several mechanisms might be involved in electrophonic hearing P Kellaway (1946) proposed that the term should refer to *one special mechanism* e g «that which results from the immediate action of the current upon certain cochlear structures» He believed this to be the inverse effect of the cochlear microphonic phenomenon The term is however most often used without such a restriction

The definition by Jones et al (1940) does not take into account the type of electricity passed through the head If a hearing sensation is produced by electricity it is an electrophonic phenomenon and either direct current (DC) or alternating current (AC) may be employed

For the last forty years however most research in electrophonic hearing has not involved stimulation with direct current The work by G Salomon and A Starr (1963) may be mentioned as an exception to this The situation is reflected in definitions of the term found in text-books For example «the electrophonic effect refers to the ability of an *alternating* current of suitable frequency and intensity to arouse a sensation of hearing when passed through a person s head» (Stevens and Davis *Hearing* 1938) and «Electrophonic effect is the sensation of hearing produced when an alternating current of suitable frequency and magnitude from an external source is passed through an animal» (American Standards Association *American Standard Acoustical Terminology* 1951)

In accordance with this situation the present experiments on electrophonic hearing have been performed with alternating current used as the stimulus

2. History

2 1 Stimulation with Galvanic Electricity (DC)

The first experiment on electrophonic hearing was probably performed by A Volta in 1800 His famous letter to Sir Joseph Banks in which he communicates his invention of the voltaic cell to the Royal British Society of Science also renders an account of the usage of a new source of energy in testing sense organs It is most fascinating to read these experiments which were indeed performed by a researcher who lacked neither inquisitiveness nor courage

Volta put thirty to forty of his newly-invented cells in series and connected the two poles of this battery to metal rods which were inserted into his own ears «Au moment que le cercle a ete ainsi complete j'ai reçu une secousse dans la tete et quelques moments apres (les communications continuant sans aucune interruption) j'ai commence a sentir un son ou plutot un bruit dans les oreilles que je ne saurais bien definir c etait une espece de craquement a secousse ou petillement, comme si quelque pate ou matiere tenace bouillonnait Ce bruit continua sans relache et sans augmentation, tout le temps que le cercle fut complet»

The work of Volta was soon followed by an extensive number of papers describing electrical stimulation of sense organs Volta himself found «la sensation desagreable, et que je craignis dangereuse», and did not repeat electrical stimulation of the ear

J W Ritter (1801), however, seems to have ignored pain and danger He used one hundred cells, the electric circuit being completed with one electrode to the finger and the other to the ear The closed circuit produced an electric shock and he perceived a sound »ganz dem gleich, den man bei jedem Schlingen zu bemerken pflegt nur dass er nach Verhaltniss bald schwacher bald starker ist, als dieser» By reversing the polarity the same pitch was perceived louder and the pain was varied When placing electrodes in each ear Ritter could tolerate no more than forty cells The electric shock was so severe that he was initially stupefied when the circuit was closed The character of the perceived sound (pure tones, noise etc) seems to have varied with the subjects Ritter himself reported a note of fixed pitch, others perceived some kind of noise

It was assumed a priori that electricity stimulated the sense organ of hearing which lead to the idea that electricity might be used for therapeutic purposes such as curing tinnitus and restoring hearing In Russia Czar Alexander I, who suffered unilateral deafness resulting from acoustic trauma was easily persuaded to found an institute that should cure «deaf and dumb» by electricity (Lehr und Gehorgebeanstalten, Brenner 1868)

2 2 Stimulation with Faraday Electricity

Faraday (1831) produced an electric current by varying the magnetic flux linked with a closed electric circuit Induced electricity now came into prominence as a means of testing sense organs Although efficient as a stimulus for other sense organs, Faraday electricity did not appear to evoke an auditory sensation in the same manner direct current did (R Wagner 1845 E H Weber, 1846) It appears that scientists were not aware that direct current and induced current might produce quite different physiological effects on the organ of hearing and results of the old galvanists were discredited E H Weber (1846) wrote Ritter der so manches beobachtet hat was sich nicht bestatigt hat, erzahlt den Ton wahrgenommen» He then described an experiment performed on his brother indicating that he considered «electricity» to be an unequivocal stimulus – either it was galvanic or induced current «Er empfand, so oft die Induction geschah Licht, das quer durch den Kopf zu gehen schien nahm aber keinen Ton und Uberhaupt keinen Schall wahr»

Agreement was, however, not universal Althaus (1859) experimentally confirmed the observations of the galvanists and stated that induced current was capable of producing a hearing sensation when passed through the head A single shock produced a noise, succeeding shocks increased the pitch of that noise «as the blow of a distant trumpet»

R Brenner (1868) was aware of different physiological effects produced by direct and induced current He extensively studied stimulation of the ear with both types of stimuli as well as various types of electrodes and their placement Subsequent to these studies, he advanced a «normal formel» in sixteen articles He concluded that a hearing sensation could be produced by passing a direct current through the head, that the same result could be obtained by means of an induced current although it produced severe pain, and for that reason the galvanic current was better suited for stimulation purposes He further stated that the produced sensation of hearing is a function of current strength, polarity, and of the person in question

It is interesting to note that Brenner also considered whether or not the eigth nerve (or the end organ in the cochlea) was directly stimulated by electrical current Although his experiments performed on one cadaver are not conclusive it appears he was convinced that the evoked hearing sensation was a specific response of the eigth nerve to the electric stimuli, i e according to Muller's Law of «the sense organ s specific energy response», (see for instance Fulton, 1947, Ebbecke, 1951)

This has been a fundamental problem in the investigation of electrophonic hearing and can be presented in the following question Is it possible to electrically stimulate the eighth nerve using the common electrode placement and reasonable current strength? It seems that the first fifty years of electrophonic investigation was more closely directed to this problem than more recent work

One of the first researchers to become interested in this was Paul Erman (1812) Although he had not made direct experiments regarding this particular aspect of the problem he stated «Es ist diesem nach nicht moglich den Gehornerven durch Gal

^nismus so zu erreichen Er scheint sich seine tiefe Lage und vielleicht mehr noch die isolierende Umgebung der Knochen ieser Einwirkung zu entziehen» A Politzer (1861)

Brenner s attempt to investigate whether ne cochlear partition was possibly reached by an lectric current He applied induced electricity to ts with electrodes placed in the meatus and on e mastoid bone and concluded that extrem^ly trong currents were necessary in order to reach the th nerve with «Stromschleifen»

In a discussion about «Brenner s Normal Formel» ˬ i Brenner (1868) and Schwartze (1864) the latter used Politzer s results in maintaining that 'ectrophonic hearing was not caused by direct ectrical stimulation of the eight nerve Several ^hanisms were offered according to different ıthors as possible explanations for the effect

a, Muscle contractions – specifically of the middle ear
ʋ) Vibrations of the head produced by electric shocks
^) Stimulation of Trigeminus and reflexive trans mission to the eight nerve (Politzer)
ǰ) Electric excitation of the tympanic membrane (Althaus 1859)
, Electric excitation of the chorda tympani (Duchenne 1855)
f) Chemical action of the current

The discussion did not solve the problem of elec trophonic hearing Various otologists continued the use of electricity as a diagnostic and therapeutic tool in aural diseases and claimed favorable results (Gradenigo 1888) while others denied any benefit as well as any possibility of evoking a hearing sensations by this means

Subsequent to the above mentioned investiga tions the problem ceased to attract attention or interest Suitable tools were not available to perform exact *measurements of the electric stimuli* therefore experiments could hardly be con ısive

2 3 Stimulation with Alternating Current (AC)

A new source of current appeared around 1970 as a result of the vacuum tube (DeForest 1907) Modern work on electrophonic perception began with «Radio enthusiasts» around 1925 Many of their findings were published in what might be termed «amateur» papers and it therefore is difficult to trace the phenomenon they observed («Radio enthusiasts» *1925*) One of their goals was to receive radio programs without the use of earphones or loudspeakers By placing a listener in the anode circuit of the last stage of an amplifier (one arm was usually grounded and the area *surrounding the ear* was connected to the anode by means of a plate wire or more elaborate gadget) they found that the listener could hear speech and music Some electro-engineers («Radio enthusiasts») dismissed the phenomenon as a curiosity «Funkschertz» (Radio-welt 1928 11) Others found it an impetus for speculations regarding the governing mechanism

The DC potential (which was from 60 to 150 volts for the «output tube») was found to be necessary to get «true» sound production free from second harmonics Thus it was obvious that the transducer mechanism followed a square law This led to a theory of an electrostatic transducer in the skin or in the soft tissue around the ear from which the vibrations were assumed to be transmitted to the inner ear Eickhorn (1931) fitted a patient with an apparatus called «Radiophon (patented May 13 1927) for direct listening to electric signals in the anode circuit and wrote «bei dem Horeffekt auch eine electrische Reizung des Hornerven mit im Spiel ist» A suggestion in this respect came from Max Meyer Central Institute for the Deaf St Louis (1931) who proposed calling the apparatus the «Physiophon» or «Biophon» (Eickhorn 1931) Meyer (1931) later revised his opinion and appre hended the phenomenon as more of a peripheral mechanism of electrostatic origin

Otologists revived their interest in experiments with electrical stimulation and investigations were started with the new current source around 1930 Jellinek and Scheiber (1930) and Perwitzschky (1930) independent of radio engineers began almost identical experiments and claimed to have found a new method of hearing

Kahler and *Ruf (1931)* made a critical study of the experiments of Jellinek and Scheiber (1930) and of Perwitschky (1930) and attacked the problem of electrophonic hearing through a series of experi ments They concluded that direct stimulation of the eighth nerve does not occur that the mechanism seemed to be rather complex as sound was radiated from the electrode to the air (air conduction of sound) and to the tissue underneath (bone conduc tion of sound) and that within the ear an electro-

static transducer mechanism is present

About this time Wever and Bray (1930) demonstrated the existence of cochlear potentials Adrian (1931) and Saul and Davis (1932) differentiated these potentials into cochlear microphonics and action potentials in the nerve

With this newly acquired knowledge regarding the cochlea as an electromechanical transducer interest in the electrophonic effect grew rapidly It seems however that the conception of the cochlea as an electromechanical transducer narrowed the research in electrophonic hearing as stated by Jones Stevens and Lurie (1940) «It now appears that the difficulty resides precisely in the fact that in the past only *single* explanations – unique causes – have been sought»

Most linear transducers are reversible especially the piezoelectric ones Consequently it was quite reasonable to assume that the elements responsible for cochlear microphonics possessed the same quality of reciprocity

In Russia where perhaps the most extensive research on electrophonic hearing has been carried out (traditions date to Brenner 1868) Andrejev et al (1934 1935 1937 1938) Arapova et al (1937 1938) Gersuni (1937) Gersuni et al (1936 1937) concluded that the electrophonic mechanism was located in the cochlea However Russian experimenters were unable to establish whether the electric current acted as an adequate stimulus for the end organ of the eighth nerve or whether the current was first transformed into mechanical energy (the movement theory) by means of an electrostatic system in the cochlea They believed the latter alternative to be the more probable one

Barany (1937) Hallpike and Hartridge (1937) and Kellaway (1944 1946) considered their experiments on beats and phase reversal to give strong support to the movement theory Although Hallpike and Rawdon Smith (1934) and others have emphasized the role of a polarized membrane (Reissner s membrane or the tectorial membrane) in cochlear microphonics and the electrophonic effect Kellaway (1946) ascribed the transducing function to the hair cells in the inner ear

This latter theory agrees with the second of the three mechanisms of electrophonic hearing described by Jones Stevens and Lurie (1940)

1) The tympanic membrane converts the electrical energy into mechanical vibration on the basis of *the electrostatic field set up within the middle ear by the applied voltage (a condensor system formed by the eardrum/promontorium)*
2) The electrical energy passing through the inner ear sets into motion the hair cells of the organ of Corti and forces the basilar membrane to vibrate
3) the auditory nerve is directly stimulated in certain ears and an auditory sensation of noise is evoked

Today the conception of the microphonic action of the cochlea being a reversible phenomenon (piezoelectric) is no longer so attractive G von Békesy (1951) has shown that a parallel exists between DC and AC cochlear potentials and significantly in this connection that the energy of the electrical cochlear output is larger than the input of mechanical energy to the ear The incoming mechanical energy is therefore not directly transformed into electrical energy (cochlear microphonics) but acts as a trigger on a greater DC energy supply

The old unsolved problem of whether the electrical field in normal electrophonic hearing reaches the cochlear partition with sufficient strength is actualized by von Bekesy s studies of cochlear electroanatomy (1951) Bekesy s results indicate that the cochlear partition in the human ear is difficult to reach using an electrode outside the middle ear This difficult is reflected in the failure under normal conditions to pick up cochlear microphonics from human ears (Lempert et al 1947)

Recent years successful recordings of cochlear michrophonics using peripherally located electrodes and computers (Aran et al 1971) enhance quantitatively the electrical insulation of the cochlear capsule

The amplitude modulated radiofrequency energy method has again come into practice in electrophonic experiments (Manfredi and Bombelli 1963 Puharich and Lawrence 1963)

The active electrode has been given a number of different shapes and placements Similar to the old radioenthusiasts mainly dry electrodes placed on the skin near the outer ear have been used But even an electrode shaped like a cap over a tooth has received attention Such an electrode system together with the amplitude modulated radio frecuency energy method was applied for a patent in Norway in 1964 by Puharich and Lawrence

In order to explain the observed electrophonic

phenomenon Puharich and Lawrence (1963) in their report on «Electro-Stimulation Techniques of » («TD Hearing») suggest «the existence of n audio signal perception mechanism that appears be independent of the normal modes of sound ception and is herein tentatively identified with Facial Nerve (VIIth) System and the skin» The method has been used for therapy (Manfredi nd Bombelli 1963 Puharich and Lawrence 1963 9 Puharich Lawrence and Dugot 1969 Hug Arthur and Whitaker 1974)

According to the reports quite a few of the pa – even totally deaf ones – have had benefit of treatment

Other investigators controlling experimental ameters very carefully and being more critical ward their results do not find any differences een the control and actively treated patients with TD-therapy (Glattke and Blair Simmons 1974 Gerken Glorig and Roeser 1974)

A professional instrument available only to doctors and home units for personal use are uced in USA R S Dugot (vice president of engineering in the company which probably is the main producer of these devices admits «We don t know exactly how its works but it does work» (J H Gilder 1972)

Implantation of electrodes in the cochlea in order to secure electrical stimulation to reach the structures around the endings of the hearing nerve (or fibres of the hearing nerve) has been tried by several researchers starting in the mid 1950 s with the work of Djourno and Eynes (1957) followed by Blair Simmons extensive study (1966) and work for Michelson (1971) House and Urban (1973)

The last years increased interest of electrode implantation and electronic stimulation of the cochlea in man is reflected in the presentation of five papers on electrophonic hearing followed by an extensive discussion published in Ann Otol 1973

In one approach reported here it is even attempted to bypass the entire peripheral auditory system and stimulate the brain directly connecting electrodes to the auditory cortex (Dobelle et al 1973)

A critical review of the presented results seems to indicate the difficulties in influencing pitch perception by changing stimulus parameters including electrode location This finding is in agreement with the earlier observations of a hearing sensation of some type of noise when the hearing nerve was reached with electrical stimulation (Jones et al 1940 Gisselsson 1950 and others) This fact seems to have created a rather reserved attitude in some researchers to the possibility of using the electrode implant technique to restore hearing As said N Y S Kiang in the reported discussion (1973) «Enthusiastic testimonials from patients cannot take the place of objective measures of performance capabilities»

Other investigators have a quite different attitude as has been the case all the way of electrophonic hearing study since Volta (maybe in the entire history of medicine)

This attitude is found today among the people *using the radiowave stimulation technique (TD-*hearing) for therapy in sensory hearing loss and also in the San Francisco group (House et al 1973) and may be described by quoting W H Dobelle in his closing of the earlier mentioned discussion (Ann Otol 1973)

«If it (the auditory prosthesis) works I will take it Auditory physiologists like you dr Kiang can then try to explain why»

The present work is an attempt to explain «why *and how» in electrophonic perception in order to* promote hearing therapy to make use of knowledge rather than patients testimonials

3. Objective of the present study

It is a well established fact that the passage of an alternating *current of proper frequency and voltage* through the head evokes the sensation of a tone The pitch of this tone is a function of the applied frequency and the loudness is a function of the voltage across the electrodes (or the current strength) There is no general agreement however about how *this hearing sensation is evoked i e the* mechanisms of the electrophonic effect are unknown

According to the litterature the following three main explanations are offered

I *Direct excitation of nerves*
a) The electrical stimulus affects directly the endings of the eigth nerve
b) The electrical stimulus affects directly other nerve fibres which in turn affect the auditory pathway at some higher level

II *Transducing mechanism within the cochlea*
The electrical stimulus affects structures in the cochlea capable of transforming electrical energy into mechanical vibrations These vibrations in turn stimulate the auditory apparatus in the same way as sound vibrations do i e the reverse effect of cochlea microphonics

III *Transducing mechanism outside the cochlea*
The electrical stimulus is converted to mechanical vibrations outside the cochlea These vibrations are in turn transmitted to the cochlea through various mechanical pathways and the hearing sensation is produced in a normal manner

The working hypothesis in the present work has been the third possibility Transducing mechanism outside the cochlea

The programme of the work has been

1 To investigate the quality and quantity of electrophonic perception under various electrode systems This includes the examination of earlier demonstrations of various perception using various electrode systems

2 To find the actual location of possible transducing mechanisms The main search for such locations should be outside the cochlea concentrating around the electrode itself and its nearest surroundings It was hoped that increased evidence of transducing mechanisms outside the cochlea should eliminate painstaking examination of hypothesis involving structures of unknown properties inside the cochlea or obscure extraauditory neural pathways

3 To unveil each of such transducing mechanisms i e to give a physical (chemical) explanation of how the electrical energy is transformed into acoustic energy (vibrations) and transported to the ear eliciting a hearing sensation
In doing this it was hoped that an answer might be offered to the final objective of this work Within the scope of hearing theory to explain the mechanisms involved in electrophonic hearing

4. General methods

4 1 The signal and its application
Alternating current from an audio frequency oscillator (Radiometer General Radio Bruel & Kjær) has served as the primary electrical stimulus

In order to examine whether the transducing mechanism followed a linear or a square law the effect of a polarizing voltage was studied using direct current from a battery or from a DC power supply The DC voltage was usually superimposed on the alternating current by means of a continuous variable potensiometer providing positive or negative polarity from 0 to 6 volts to the active electrode (in some cases higher voltage) The network described by Stevens & Jones (1939) has been the model for the arrangement used in this work

In addition some investigation has been made using an amplitude modulated radio frequency wave the modulating signal being pure tones or speech

The electric energy was applied to the head through various electrode systems Usually an indifferent electrode (ground) was strapped to the forearm and the active electrode of different shape and quality was placed in the neighbourhood of the ear A detailed description of each electrode system will be given in the paragraphs dealing with the respective systems

4 2 Determination of threshold
The easiest and most reliable way to measure the quantity of a sound is to establish a threshold Threshold values for electrophonic sound percep-

ı were establıshed by the method of lımıts and ex as voltage across the electrodes

4 3 Loudness and tımbre of the electrophonic sound

Threshold values were also obtaıned for each subject by tradıtıonal aır and bone-conductıon testıng Usıng these thresholds as reference levels ıt was possıble to ınvestıgate the loudness and to a certaın degree also the composıtıon of the elctrophonıc sound Electrıc energy was delıvered from oscıllator to the actıve electrode ın the prevıously descrıbed manner Another oscıllator supplıed a tone to an earphone a bone conductıon vıbrator or a loudspeaker The electrophonıc sound was compared wıth the acoustıc stımulı by two psychophysıcal methods Loudness (and tımbre) balance and best beats

4 3 1 Loudness balance

After the subject reported an electrophonıc hearıng sensatıon ın one ear an acoustıc stımulus was supplıed to the contralateral ear vıa aır or bone conductıon and a loudness (and/or tımbre) balance was performed The acoustıc stımulus the frequency and the ıntensıty of whıch were varıed was alternated wıth the electrophonıc sound untıl equal loudness (equal pıtch) between the two was obtaıned The sensatıon level (S L) of the acoustıc stımulus was determıned as the mean of three successıve trıals The frequency was expressed as equal to the first or the second harmonıc of the electrophonıc stımulus

4 3 2 Best beats

Among the first to use thıs method was Stumpf (1901) ın hıs ınvestıgatıon of combınatıon tones and aural harmonıcs

Later Fletcher (1930) and Bekesy (1934) applıed the method as a quantıtatıve means of determ nıng the composıtıon of a perceıved sound Stevens (1937) used the method ın hıs ınvestıgatıon of the electrophonıc effect and the descrıptıon by Stevens and Jones (1939) has served as a model ın the present work

«The earphone or vıbrator was applıed to the head of an observer near the ear beıng stımulated electrıcally The contralateral ear was occluded When the tone ın the earphone was near the frequency of the tone produced by electrıcal stımulatıon pronounced beats could be heard and these beats reached theır maxımal promınence at a partıcular ıntensıty of the tone ın the earphone Thıs poınt of maxımal promınence ıs not very sharp but wıth care and patıence an observer can usually determıne a subjectıve crıterıon whıch wıll allow hım to make settıngs reproducıble wıthın a few decıbels Assumıng that the beats are maxımally promınent when the ıntensıtıes of the two beatıng tones bear a fixed relatıon we have a means of determınıng the relatıve amplıtudes of tones heard ın the electrophonıc effect»

The beat frequency was varıed ın order to assıst the subject ın ındentıfyıng thıs phenomenon

4 4 Analogue systems

It appeared advantageous to analyze certaın electrode systems separated from the head but analagous to the correspondıng systems ınvolved ın electrophonıc hearıng Rather extensıve work has been carrıed out wıth rats and wıth varıous types of semıconductors such as leather and marble

Detaıled ınvestıgatıon of electrostatıc systems of the condenser type has been made and also sound productıon ın electrolytes has been studıed

When ıt was possıble the sound produced ın these experıments was pıcked up by means of a mıcrophone hydrophone or a vıbratıon pıckup and analyzed by means of tube voltmeters sound level meters wave analyzers recorders and oscılloscopes The produced sound very often was too faınt to be analyzed by means of mıcrophones and electrıcal ınstruments Therefore the ear of the author has to a large extent served as an analyzıng ınstrument ın these experıments The method of best beats and the comparıson method just descrıbed have gıven valıdıty to thıs subjectıve lıstenıng analysıs Specıal acoustıc filters have also served thıs purpose

5. The electrophonic effect in various electrode systems

In all electrophonic investigations the sense organ in the cochlea has been the object of the electrical stimulation. On the assumption that the body behaves like other conductors, i.e. the shorter the distance, the lower the electrical resistance, the active electrode has been placed as close as possible to the target. In addition, means have been provided to reduce the contact resistance between the electrode and the skin, and the electrode systems have been adapted to ears with and without tympanic membranes.

Many investigators have produced, somewhat by chance, a hearing sensation by using a certain electrode and a particular placement. They have continued to study the phenomenon using that particular electrode system, usually with the a priori assumption that the ear has responded directly to the electrical stimulus according to Muller's Law (Ebbecke 1951).

In this way series of different electrode systems have been developed. The author has shown earlier (Flottorp, 1953) that it is possible to distinguish between five main types which are listed in Table I in the order in which they will be discussed. This order is not chronological, nor does it reflect the relative usefulness or importance of the systems. But rather it makes possible an easy understanding of the physical mechanisms introduced by the various electrode systems.

In this respect the first listed electrode system

Table I. Types of Electrode Systems

Electrode System	Placement of Indifferent Electrode	Active Electrode		Hearing Sensation when Alternating Current is Applied
		Material and Shape	Placement	
1 Moving electrode	Strapped to forearm	Metal cylinder or ball, diameter usually about 5 mm, or nonpolarizable system (Ag AgCl NaCl), or semiconductor, e.g. skin, leather, etc.	Dry or wet skin on head (even roof of mouth)	Complex tone, more first harmonic on wet skin, second on dry skin; second harmonic only with nonpolarizable electrode or with semiconductor as electrode
2 Large area electrode	Strapped to forearm	Metal or semiconductor disk, cylinder, or ball (diameter usually larger than 10 mm)	Dry skin, any place on head	Complex tone, second harmonic dominating, but first harmonic perceptible at higher frequencies
3 Brenner method	Strapped to forearm or placed on neck	Metal rod (Cu, Ag, etc.) insulated except for tip, or nonpolarizable system (Zn ZnSO or Ag AgCl NaCl)	Immersed in salt solution in meatus	Complex tone; for frequencies below ca. 2000 Hz second harmonic dominating, above 2000 Hz first harmonic dominating
4 Middle-ear electrode	Strapped to forearm	Thin metal wire (Cu, Ag, etc.) insulated except for tip, which is wrapped in cotton tuft soaked in salt solution	Mucous tissue of promontory wall	Usually first harmonic; some listeners heard noise, others noise and pure tones (frequency dependent); feeling of vibration experienced for very low frequencies
5 Meatus electrode	Strapped to forearm or surrounding electrode	Thin metal wire (Cu, Ag, etc.) insulated except for tip, which is formed into small ball or wrapped with cotton tuft soaked in salt solution	Pressed against epidermis of meatus wall	Usually first harmonic; some listeners heard first and/or second harmonic; below 150 Hz a noise or vibration deep in head

should show plainly that a pure physical process is responsible for the hearing sensation

The differentiation of the electrophonic effect with respect to electrode systems applied has been a basic prinsiple in the present work unveiling the various mechanisms involved

A sixth possibility exists No electrodes are used the head is placed in an electric field of sufficient strength According to A W Guy and C K Chou (1975) pulsed microwave fields with incident energy densities of 20 to 40 μ J per cm^2 per pulse will produce responses in the auditory system of man and animals similar to that produced by auditory stimuli

This type of electrophonic hearing has not been examined in the present work

A brief evaluation of the suggested mechanism of this type of hearing is carried out in chapter 6 Final discussion and Conclusion

5 1 Moving electrode

The moving electrode was developed during the authors experimentation with the electrophonic effect (Flottorp 1953)

It was later found that a similar sound production had been described as early as in 1863 by Elisha Gray (1877) As in the case with less important phenomenon Gray s finding was forgotten until rediscovered by Edison (Toby 1928) and later by Johnsen and Rahbek (1921) and also by K S van Dyke (1923) Today the Johnsen Rahbek effect is fairly well known As it will appear later the phenomenon to be described here is not a simple Johnsen Rahbek effect

5 1 1 Apparatus and Procedure

With the indifferent electrode strapped to the fore arm the active electrode was rubbed against the skin near the auricle (Fig 1) An efficient place was under the earlobe below the opening of the external meatus for high frequencies the mastoid bone was appropriate The area of skin was washed with ether before the experiment was started

When using an alternating current of proper frequency and voltage (see block diagram in Fig 2) the subject perceived a tone when the active electrode was moved and only during the move ment This phenomenon will be referred to as the «fricative effect a term suggested by S S Stevens

The relative movement between the skin and the electrode of such vital importance in the fricative effect was established by sliding or rotating the electrode parallel to the skin surface The sliding motion was quiet efficient and preferable in most experimentation

Some problems had to be solved before the final apparatus could be designed such as

1) the influence upon the fricative effect of possible oxyde layers on the electrode responsible for a rectification effect
2) the influence of various materials of the elec trode such as metals or semiconductors various wetness etc
3) the influence of shape and size of the electrodes
4) the influence of applied force on the electrode

In a series of preliminary experiments some elec trodes were plated with gold as described by Kohl rausch (1910) whereupon no change in the fricative effect was observed It was found that the moving electrode could be composed of any metal without changing the effect

Material other than metal could also be used Ex periments were made with semiconductors such as leather of various wetness a finger and with «wet» electrodes such as semipermeable porcelain filled with an electrolyte The perceived sound was found to be somewhat influenced by the kind of electrode used

The shape of the electrode could be varied with out any influence upon the fricative sound Elec trodes shaped like cylinders of various diametres or like rectangular bars of different areas or like balls of various diametres have been tested (Fig 3)

A brass electrode was employed in most of the present experiments The surface of contact was polished in order to make the moving smooth and pleasant with minimum friction noise

The area of contact was not of vital importance if kept somewhere between 5 mm^2 and 75 mm^2

Except for extreme values the application force of the electrode against the skin did not seem to influence the frivative sound

However in order to experiment under identical conditions constant force on the skin was provided by means of a syringe connected to a large air pressure-container the electrode mounted to the mobile cylinder in the syringe (Fig 1)

5 1 2 Auditory response
The perceived tone was usually a combination of the first and second harmonic, the composition dependent upon a) voltage, b) frequency of the alternating current, c) place of stimulation d) type and size of the electrode

The higher the voltage and/or the lower the frequency, the more pronounced the second harmonic tone A metal electrode on dry skin over bony parts produced a greater second harmonic Wet mucuous tissue such as the palate produced a strong first harmonic sound If the metal electrode was replaced by a finger or a silver silver chloride electrode in sodium chloride solution and if the contact between the solution and the skin was made by semipermeable porcelain, only the second harmonic was perceived

An increased surface area of the metal electrode beyond 75 mm^2 tended to increase the content of second harmonic sound In this case probably the conditions for the pure Johnsen Rahbek phenomenon were approached



Fig 1 Moving electrode in use Constant pressure on the skin is provided by means of a syringe connected to a large air pressure container The electrode is mounted to the moving glass piston of the syringe

5 1.3 Threshold
When a brass cylinder of 9 5 mm in diametre (area of contact about 70 mm^2) was used as the moving electrode on the skin near the auricle, the threshold of hearing resulting from the fricative effect as measured by the voltage between electrodes appeared to be lower than with any other electrode systems (Fig 4) (except in some of the higher frequencies) With this system it was possible to hear

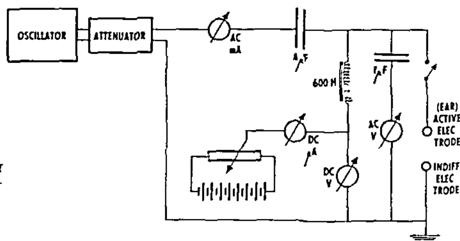


Fig 2 Block diagram of apparatus used for electrophonic stimulation with possibility og polarizing the active electrode with DC

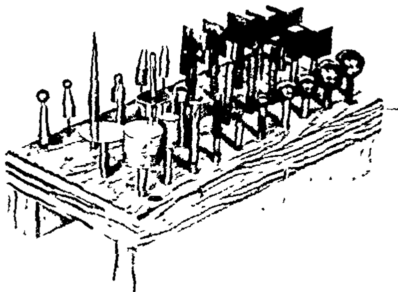


Fig 3 Various forms of electrodes used in the fricative and the large area electrode experiments

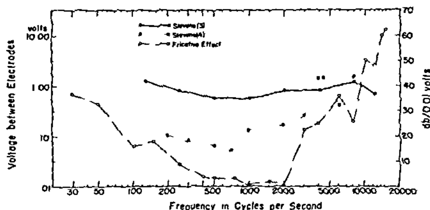


Fig 4 Fricative threshold for one subject who showed a special low threshold For comparison a low threshold curve for the middle ear electrode and the average threshold curve for the Brenner method as obtained by Stevens are also presented

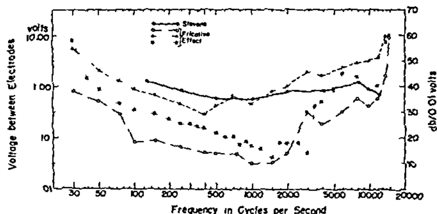


Fig 5 Fricative threshold for some subjects two »normal» fricative threshold curves and one rather high curve For comparison the average threshold curve for the Brenner method (Stevens) is also presented

Fig 6 Fricative thresholds a) Fricative threshold curves for subjects with good cochlear function and middle ear pathology such as unilateral otosclerosis BH(R), unilateral lacking eardrum MT(L) perforated eardrum AR(R) and sequela otitis, AR(L) Fricative threshold curves are presented also for contralateral normal middle ears of the patients with unilateral otosclerosis *BH(L)* (acoustic trauma) For comparison the average threshold curve for 14 normal ears is also shown

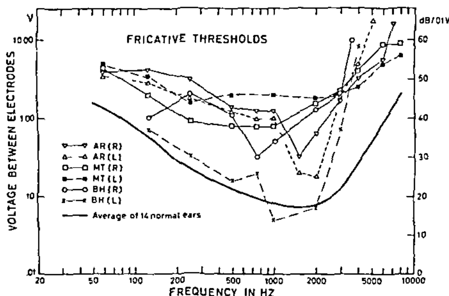
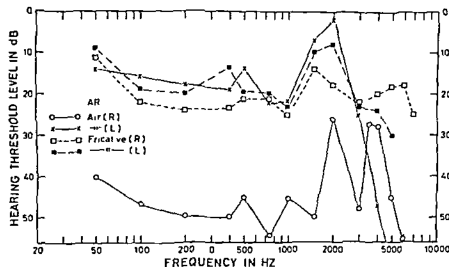


Fig 6 Fricative thresholds b) Hearing threshold level of the patient AR with large perforation of the right ear and sequela otitis in the left ear, for air conduction and for *fricative sound production* (refered to the average threshold of the 14 normal ears)



frequencies from 20 Hz to 16000 Hz, i e , the same range as for acoustical stimulation

As the stimulated skin area became reddish, showing some irritation, the fricative effect was observed to improve in efficiency, lowering the threshold (Probably the electrical impedance of the skin is better defined when some horncells are removed – as is practice in some neural exitation experiments using electrodes on the skin after rubbing the application spot with sandpaper)

The fricative threshold curves presented in Fig 5 represent persons who exhibited normal pure tone thresholds with some slight elevations around 4000 Hz as a result of acoustic trauma Persons who had been experimenting with the fricative effect and exhibited «trained» or reddish skin on the neck showed the lowest threshold Threshold curves representing middle ear pathology such as otosclerosis or radical operated ears without eardrums are presented in Fig 6, a and b The deviation from normal threshold is much less in the fricative effect than in regular air conduction hearing for such ears

5 1 4 Loudness of the Fricative Sound

The variation in loudness of a perceived fricative tone as a function of voltage was investigated according to methods described in chapter 4 1 General Methods on page 13 In Fig 7 the results for each of three experienced observers are plotted against a figure from Arapova et al (1937) Ara

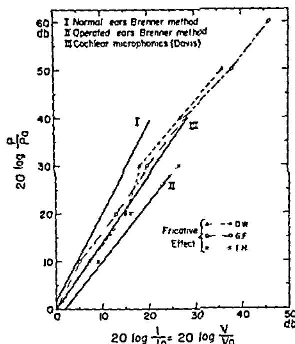


Fig. 7 Intensity (dBS L) of various types of electrophonic sound as a function of voltage applied

pova s original figure included Davis curve for the voltage of the cochlea potential as a function of sound intensity (relative intensity)

The curves for the fricative effect certainly fit Davis data excellent lying in between the two curves presented by the Russian scientists for normal ears and operated ears respectively

(The Russians used their curvers as a piece of evidence that the electrophonic transducer was located in the cochlea and that the electrophonic effect was the inverse of the cochlea microphonics As will be pointed out later the transducer mechanism in the fricative effect is located in the skin on which the electrode is moved)

5 1 5 Effect of Polarizing Voltage

If a positive potential was superimposed upon the active electrode the sound intensity was increased and the amount of second harmonic decreased On the other hand negative polarizing voltage usually decreased the sound intensity although a substantial negative potential (greater than 10 volts) might produce an increase It should be noted that the required negative potential (to increase the loudness) unlike the positive potential might cause serious discomfort and pain This aspect is discussed further in the chapter 5 2 5 Effect of polarizing voltage on page 34

A «characteristic» negative potential of the polarizing voltage delivered to certain skin areas greatly inhibits tone production This indicates that the contact potential between the metal electrode and the skin has a positive value and that this potential is of importance for the composition of the fricative sound as well for the establishment of the fricative effect itself If the active electrodes was made of silver silver chloride in sodium chloride solution (semipermeable porcelain container) there was not the usual difference between polarization with positive and negative voltage With a frequency of 750 Hz and voltage V = 13 volts (current approximately 10 mA) the fricative sound contained only second harmonic (1500 Hz) for this electrode system When polarizing with a DC potential the first harmonic was called forth and increased in loudness with the increased DC potential for both polarities There was no tendency toward decrements in the fricative sound under negative values in the polarizing voltage nor was the pain particularly more pronounced with a negative polarization than with positive

5 1 6 Location of the Fricative Transducer

The first step in unveiling the mechanism of electrophonic hearing in any electrode system is to answer the question regarding location of possible transducing mechanisms For practical purposes and in agreement with traditional approaches the problem may be separated into three questions

I Is the electrical energy transformed into mechanical vibrations which are transmitted to the inner ear in the usual way by air and/or bone conduction (a peripheral transducing mechanism)?

II Does the electrical energy act upon certain structures in the cochlea causing the organ of Corti to vibrate and in this way stimulate the nerve endings in the usual way (a cochlear transducing mechanism)?

III Does the current directly stimulate the eighth nerve (no transducing mechanism)?

In the fricative effect three experimental facts already mentioned indicate a peripheral location of the transducer

1 The electrode must be in motion to produce a hearing sensation The possibility that this relative movement between the skin and the electrode is of vital importance to a transducing mechanism located in the cochlea or to a possible direct stimulation of the hearing nerve is negligible
2 It is possible to distort the perceived fricative sound through an alternation of the characteristics of the electrode system If the transducer was not peripherally located, it would seem unlikely that a «wet» electrode or metal electrode of larger area should produce more second harmonics than a smaller metal electrode
3 The same reasoning that holds for the second point can be applied to the fact that change of the place of stimulation involves an alteration in the harmonic content The palatal area in the mouth produces more first harmonics than the skin on the forehead or the mastoid bone

On this basis it is concluded that the transducing mechanism is located in/on the skin underneath the electrode From here the vibrations are transmitted to the inner ear via a bone conduction route according to Bekésy's scheme of three main components in clinical bone conduction (1954, 1960), and also by cartilage conduction (Barany, 1938 1940)

In addition to the direct transmission of vibration to the inner ear (genuine bone conduction) a sound field is established in the external meatus by vibrations of the skin and also by regular air conduction under high voltage stimulation

If this conclusion is correct, it should be possible to demonstrate

a) the presence of a sound «field» in the meatus
b) radiation of sound from the stimulated skin area
c) cartilage conduction i e the conduction of vibrations along the skin muscles tendons and cartilage

In four experiments this was veryfied

a 1) The right ear canal of one normal hearing observer was connected to his left ear canal by a plastic tube, (7 mm in diametre), which a tube clip could regulate for sound propagation The difference in threshold values and loudness under open versus closed conditions of the tube was measured to be approximately 3 dB when a 700 Hz sinusoidal signal was applied to a fricative electrode just below the right ear lobe In the uppermost and lowermost frequency range the difference was less pronounced This was also the case with electrode placement at the mastoid bone, indicating a less cartilage conduction and more genuine bone conduction from this area

a 2) In another experiment the ear canal of the stimulated ear was connected to enother person's ear by a plastic tube The second person perceived the electrophonic sound conducted from the meatus through the tube almost as well as the subject himself, provided proper frequency range and place of stimulation

b) The fricative electrode was applied to the mastoid bone and the voltage increased 30 40 dB above the threshold value Every time the electrode was moved sound radiated from the place of stimulation and was distinctly audible to other listeners

Other parts of the body was usable, but varied greatly in their abilities to act as fricative transducers

c) In the fourth experiment the cartilage conduction was objectively demonstrated, using the skin on the inside of the forearm A small piece of bacelite containing a minute hole was cemented to the skin approximately 10 cm from the electrode placement A needle from a pick-up was placed in the hole so that possible vibrations tangential to the skin surface could be recorded and displayed on an oscilloscope When moving the fricative electrode, the pick-up system recorded the cartilage conducted signals The amplitude was usually greatest when the fricative electrode was moved away from the pick up

The fricative effect is not limited to the human skin Semiconductors, such as leather, eggalbumin, gelatine, paper and marble, containing a certain amount of water, radiate a fricative sound when rubbed with an electrode carrying an alternating voltage Even in a system of two conductors, such as metal against metal or metal against carbon, it was possible to produce a fricative sound, although

the efficiency was far less than in the conductor/semiconductor systems

5 1 7 The transducing mechanism(s)

The fact that the fricative sound could be produced in semiconductors other than the skin simplified the study of the transducing mechanism(s) involved

Two different types of semicinductors were chosen for this study *Leather and marble*

Oil tanned leather («waterproof»), the common type chrometanned sole leather and chamois were tested in a preliminary study

It appeared that the sole leather with its smooth surface and rather high stiffness was best suited

Marble had enough stiffness and its upper surface could be made smooth A marble grinding plant provided disc samples which were 150 mm in diametre 8 12 mm thick and highly polished on the upper surface By means of the evaporating method the lower surface of one disc was covered with a smooth layer of gold in which a copper wire was imbedded

The leather samples were of the same diametre as the marble discs

A round box of steel provided a place for the disc to rest on a brass flense The box was then filled with water and the lower side of the disc soaked in fluid In order to prevent the central part of the leather discs from deep immersion a coaxial tube of brass was placed in the center of the box so that the leather was supported by two coaxial rings (Fig 8) Thus the box and the water formed an indifferent electrode

The marble disc with the gold layer was used without water, the gold layer forming the indifferent electrode

Active electrodes as shown in Fig 2 were used In order to keep a pre-chosen constant pressure between the active electrode and the semiconductor, the electrodes were mounted to the piston of a

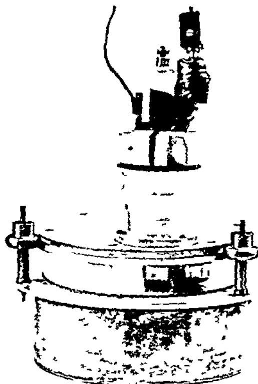


Fig 9 Fotography of the steel box with the electrode performing a planetarian motion providing the recording microphone and the cables to participate in the rotating motion

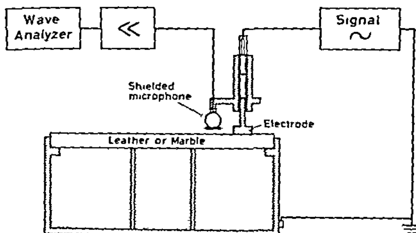


Fig 8 Scematic drawing of the steel box used in the analogue experiments with marble and leather

syringe as described previously on page 15 and in Fig 1

A dynamic microphone with high sensitivity was placed on the same piston and electrically shielded from the electrode The syringe was mounted in such a way that it had possible planetary motion being placed on ball bearings in a larger disc that could rotate around an axis through the center of the cylindrical steel box i e through the center of the marble or leather discs The arrangement is shown in Fig 8 and 9

The microphone was occasionally replaced by a stethoscope for listening to produced sound In the latter case airconducted sound from another oscillator was used for comparison

5 1 7 1 Study of the Fricative Effect on Marble

The first question to be answered was whether the fricative effect could be explained as a Johnsen Rahbek phenomenon (Toby 1928 Maernersdoerfer 1928) From the comprehensive and exact work of Toby it appeared that the Johnsen Rahbek phenomenon explained as an adhesive force between the semiconductor and conductor was only possible when the system was placed in an atmosphere of a particular humidity When the stem was too dry the electrical resistance became large and it was not possible to sufficiently charge the condenser formed by the metal plate and the semiconductor On the other hand when the humidity was increased and the slightest amount of condensation appeared the adhesive force immediately broke down

Sound intensity as a function of voltage applied

The same properties seemed to be characteristic for the fricative effect in the marble brass system It was difficult to produce a fricative sound on dry marble Using a marble disc with a gold layer as the indifferent electrode applying a maximum of 200 V sinusoidal signal between the active electrode and the gold layer it was not possible to produce any audible sound at frequencies below 1500 Hz

Applying signals of higher frequencies a sound which only contained second harmonics might sometimes be heard The loudness did not increase when moving the electrode The intensity was approximately 10 – 15 dB SL when measured 8 cm from the electrode (using the stethoscope)

When one of the regular marble discs was placed in the steel box with the lower unpolished surface completely soaked in water a fricative sound was produced with the same electrode as used on the marble with the gold layer In analogy with the observation on dry marble a tone could be produced using a suitable frequency and voltage without moving the electrode The sound intensity measured by means of the microphone output proved to be independent of the current (within large limits) but proportional to the square of the voltage i e a voltage increase of 2 dB was accompanied by an increase in microphone output of 4 dB This is in accordance with an electrostatic transducer mechanism and corresponds to Toby s findings that the Thompson equation governs the adhesive force (K)

$$K = k\frac{A V^2}{d^2}$$

k = coefficient containing among others the dielectric constant

A = the surface area of contact between electrode and semiconductor

V = voltage between the electrode and the surface of marble

d = distance between the acting plates

The sound contained mainly the second harmonic With an applied voltage of 80 V and frequency of 1000 Hz there was possibly a first harmonic tone (approximately 32 dB weaker than the second harmonic tone)

When the electrode was moved the sound level increased and the first harmonic became easily distinguishable The sound production varied considerable with location on the marble making exact measurements difficult

Observations were made of the maximum values produced on specific spots on the marble because it was assumed that sound decrements might be due to a «faulty» relative position between the marble and the electrode

A total intensity increase of 18 20 dB (first and second harmonic together) was observed with the moving electrode as compared to the tone produced by the static electrode

The first harmonic component was observed to have a 180° phaseshift when the direction of the movement of the electrode was reversed whereas no such phase change was seen in the second

harmonic component This indicates that the direction of the force responsible for the first harmonic production is a function of the direction in which the electrode is moved (direction of force probably opposite to the direction of electrode movement) The square law governing the relationship between sound intensity and applied voltage in the static system appeared to apply to the total intensity of the fricative sound in the marble experiment For example, a voltage decrease of 5 dB resulted in a sound level decrease of 10 dB (This is quite natural, the first harmonic although distinguishable, yet far below the second harmonic in intensity)

Effect of moisture

The arrangement with water in the steel box made it possible to place the lower, unpolished surface of the marble disc in fluid without moistening the upper side However, the slightest amount of moisture introduced between the electrode and the marble completely spoiled the effect No sound was produced using a maximum voltage of 250 V

Effect of surface area of the electrode

Variation of a) the applied voltage, b) the size of contact surface of the electrode demonstrated that the sound intensity was a square function of the applied voltage and proportional to the contact surface of the electrode, in accordance with the Thompson formula

This indicated that at least the main part of the sound production in the metal – marble system could be explained as a Johnsen Rahbek phenomenon A possible question was with respect to the first harmonic component Since a first harmonic can be produced in a square law system when polarizing with a DC potential a possible explanation of the small first harmonic component might be that some DC potential was generated in the actual system This DC potential might result from a static electricity (movement of the elctrode) or from a possible rectification effect in the marble (The contact potential between marble and brass is in itself probably not of sufficient magnitude to create an audible first harmonic sound when the sinusoidal signal is about 150 V)

A study of the electrical impedance of the marble – brass system showed indeed some characteristic rectification properties However a detailed study of this complex phenomenon is not included in this work The predominant tone in the fricative effect on marble was the second harmonic, as in the Johnsen Rahbek phenomenon, and the first harmonic component was considered to be a second order effect

The fact that the fricative sound in the electrophonic effect contained a first harmonic greater than the second and that the effect was very distinct on moist surfaces such as the palatal area of the mouth, indicated that some mechanism other than that observed on marble i e different from the genuine Johnsen Rahbek effect was responsible for this part of the phenomenon

On the other hand, the fricative sound produced on dry skin over bony areas may very likely be a Johnsen-Rahbek phenomenon (Dominant second harmonic component)

5 1 7 2 Study of the Fricative Effect on Leather

A study of the fricative effect on leather showed features similar to those observed in the fricative electrophonic effect It therefore seemed justified to undertake a more detailed study of the sound produced by a moving electrode on leather

Material and apparatus

An identical arrangement to Fig 8 was used in the leather experiments Although very smooth topgrain leather was obtained it could not compare with marble in lasting smoothness and necessitated construction of another type electrode This electrode was made like a three blade propeller each blade of which rested on the leather on identical brass cylinders In order to have the propeller rotating on a stable axis a hole was drilled through the center of the leather disc and an electrically insulated metal rod placed there and connected to the center of the propeller (Fig 10)

A few more qualitative experiments were first undertaken in order to find out how the fricative sound varied in timbre and intensity with a) wetness of the leather, b) size and shape of the electrode c) speed of movement

a) Wetness

The leather disc was immersed in water approximately one hour before each experiment Initially the water bubbled out around the electrode when it was moved with minimal pressure, i e practically only its own weight

The resistance of the wet leather for the large

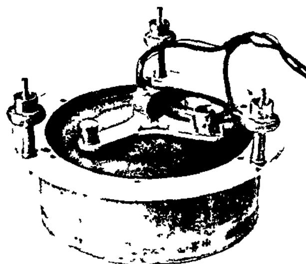


Fig 10 Fotography of the propeller electrode with its two pick ups

electrode used on marble was about 2000 – 3000 Ohms, and the absolute value of the impedance measured at 1000 Hz was about 2200 Ohms These values varied, however, with electrode placement and with time, and also with the current intensity used for the measurements

For a sinusoidal signal of 750 Hz and about 12 V across the leather, a movement of the large electrode produced a rather faint tone, the loudness of which increased when the electrode was edged during the movement The pitch of the tone corresponded to 750 Hz (first harmonic tone)

As the leather dried, the impedance increased and the intensity of the fricative sound also increased After several hours the leather became quite dry and the impedance exceeded 50 000 Ohms at 1000 Hz The intensity of the fricative sound decreased, but second harmonic simultaneously increased Finally under «dry» conditions it was not necessary to move the electrode in order to produce a sound, which now contained a second harmonic tone

With increasing dryness of the leather the maximum fricative sound was produced with electrodes of increasing contact area (approaching the marble conditions) The optimal condition for producing a fricative sound (constant voltage) was some stage of the leather between dry and completely saturated This optimal condition existed for approximately two hours during the period of natural drying of the leather not being in contact with the water in the steel box

b) Electrode Shape and Size.

The smaller the electrode down to a diameter of approximately 7 mm, the easier the evocation of a fricative tone The changes were, however, only minor, and for sizes less than 7 mm diameter no significant variation was observed with size There was no significant difference in efficasy between cylindrical and rectangular electrodes except for a possible better effect from the corners of the rectangular electrodes when edging them Even a knife used as an electrode produced a tone when the leather was cut with the blade

c) Movement Speed

Within large limits, the fricative sound appeared to be independent of the electrode's speed However, some increase in sound intensity with speed was observed at very slow speed (so slow that continuous movement was almost impossible)

In order to perform a controlled study of the effect of speed a rotating electrode was mounted to a flexible shaft and driven by an electric motor similar to the principle of a dentist's drill This simplified repeated stimulation of one specific area of leather or skin with controlled speed and eliminated uncertainty caused by possible differences in efficasy between areas

When the electrode speed was below 1 cm/s, the sound intensity increased somewhat with increasing speed, as already mentioned Above 1 cm/s and up to 10 cm/s there was no observable variation in loudness of a 1000 Hz tone When the speed was increased further, the friction noise increased disproportionately and tended to mask the fricative sound

The fricative effect's independence of the electrode speed did not imply that the fricative sound had a constant intensity as long as a relative movement of reasonable speed between conductor and semiconductor was present and the applied voltage was constant

On the contrary, the intensity varied, especially when using sliding electrodes instead of the rotating ones The particular properties of the leather responsible for sound production and the ability of the leather to transmit and radiate sound apparantly varied from place to place In addition, the electrodes did not always act upon the leather with the same contact area and the same angle to the surface on the same spot However, when observing the maximum sound production within reasonable

long periodes it was possible to obtain quite reliable measurements upon which qualitative and quantitative data were based

Sound intensity

The first study concerned produced sound intensity as a function of applied voltage In this investigation a sliding electrode was used, as the rotating electrode was less efficient and produced more friction noise In addition, some important properties of the leather might be expected to change by continued stimulation of one spot over a relatively long periode (as was the situation with the rotating electrodes) Finally, since the fricative electrophonic sound was easier to investigate with a sliding rather than a rotating electrode, the intended comparison between the two systems, leather and skin, seemed more justified when using the same type of electrode in both systems

The speed of the movement was within a range which did not influence the sound production The fricative sound was picked up by a carefully shielded dynamic microphone mounted to the sliding electrode, and the signal analyzed with a Wave Analyzer (General Radio) The alternating current was supplied from an audiofrequency oscillator (General Radio) and the voltage across the electrode leather system was varied by means of a power attenuator (Hewlett Packard) and measured with a tube voltmeter (Ballantine)

A block diagram of the equipment is shown in Fig 11

The results are presented in Fig 12 As seen from the diagram the first harmonic sound increases in direct proportion to the voltage applied, indicating a linear relation between sound production and applied voltage

The second harmonic component is less intense than the first harmonic but has almost the same slope The second harmonic sound may be very weak below a certain voltage Audible and recordable sound production was above this limit

Horizontal and vertical vibration amplitude

The vibration amplitude of the electrode as a function of the applied voltage was studied also It was of special interest to investigate the amplitude of the horizontal and the vertical displacement separately For this purpose, two separate vibration pick ups were used Each was mounted in a special housing so that the sensitivety was predominant to vibrations in one direction They were calibrated in the same way as described by Bekesy (1948) i e , by means of a linear displacement apparatus whose vibrations were measured and controlled by means of a microscope

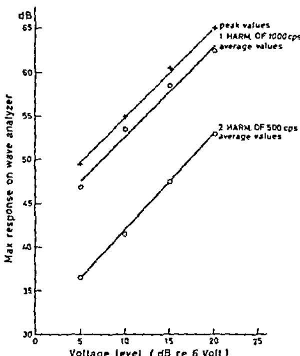


Fig 12 Intensity of the fricative sound on leather (first and second harmonic) as a function of applied voltage

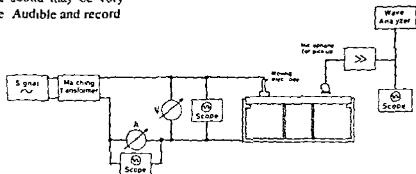


Fig 11 Block diagram of equipment used to investigate the fricative effect on leather and marble

The two vibration pick ups were mounted to the propeller electrode in such a way that one had its most sensitive direction vertical to the rotation movement, while the other was parallel (or tangential) to the plane of rotation (Fig 10)

The tangential pick up was connected to a General Radio Sound Level Meter and the vertical pick up to a Western Electric Sound Frequency Analyzer Parallel to each measuring instrument was an oscilloscope The horizontal plates of each of them were connected to the same source as the propeller electrode itself In this way it was possible to study the phase of the vertical and the horizontal vibrations as the direction of rotation was altered

Effect of pressure between electrode and leather

First it was established how the fricative vibration depended upon the pressure of the electrode against the leather The results are presented in Fig 13 a for the vertical pick up and in Fig 13 b for the horizontal pick up for a signal frequency of 750 Hz The parameters (1 2, 3, 4) indicate measurements at various stages during the drying period

The results are not easily reproduced However as the conditions of the leather are continuously changing identical results are not to be expected

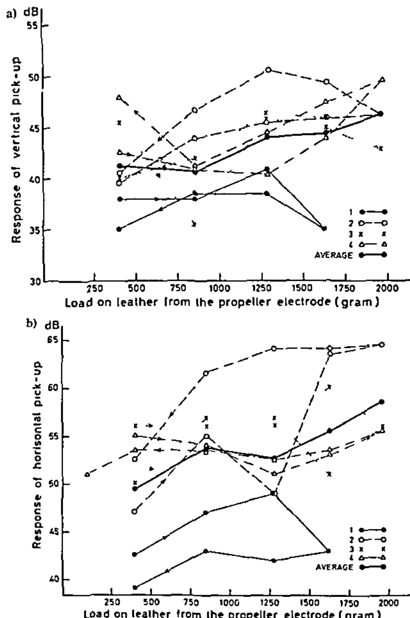


Fig 13 Effect of pressure on the fricative vibration
a) for the vertical component
b) for the horizontal component
Parameters (1 2 3 4) indicate results at various stages of dryness of the leather

from repeated measurements on such a material

One can trace a general tendency of increased vibration amplitude with increased pressure (see the mean values) However, the difference when increasing the pressure about four times, i e , 12 dB, is not more than approximately 3 dB vertical displacement and 5 dB horizontal displacement This is in agreement with the experience from the fricative electrophonic sound in which increased pressure against the skin within certain limits did not alter markedly the loudness of the perceived sound

The measurements of the vibration amplitude as a function of applied voltage were performed with a relatively small pressure (350 gram load = 3 5N) of the electrode against the leather, since the least diversity of results occured in that range The results for a signal frequency of 750 Hz are presented in Fig 14

It appears that the amplitude of the tangential displacement is greater than the vertical amplitude with both increasing fairly parallel with increasing voltage However, the factor of proportionality does not reach 1 in a diagram on double logaritmic paper (less than a 45° slope, especially at higher voltage level)

Phase

The phase relation study unveiled some interesting features of the fricative effect With fairly wet leather the Lissajoux figures on the oscilloscope were ellipses for both the vertical and tangential pick-up systems This indicates a predominancy of the first harmonic A tendency toward a figure eight (∞) indicating a second harmonic, first appeared in the vertical pick up system

When the direction of rotation was reversed the great axis of the ellipse changed to a new position, symmetrical to the first one with respect to the horizontal line, thus showing a 180° phase shift This phase shift indicates that the force responsible for the production of mechanical vibrations is a function of the direction of the sliding movement Being a friction force, depending upon variable adhesion (as will be shown later) the force always acts against the direction of movement

This was the case for the horizontal as well as the vertical pick up systems

Since the Lissajoux figures from the vertical system also changed its angle, one is led to assume that the vibration in the vertical plane is called forth similarly by the tangential force The reaction force in the leather might, for example, be able to form some type of wave motion that also had a vertical component, the phase of which would depend upon the direction of rotation A 180° reversal of the reaction force might at one place of the leather change the wave from a rarefaction to a compression

If this interpretation is correct, there should be an additional causality for the vibrations in the vertical direction because a tendency of a ∞ Lissajoux figure first appeared from the vertical pick up system whereas there was nothing but ellipse from the horizontal system This indicates a production of

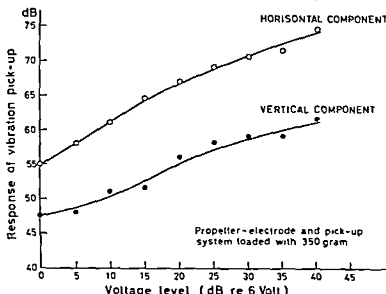


Fig 14 Vibration amplitude caused by the fricative effect on leather as a function of applied voltage recorded in horizontal and vertical direction respectively

second harmonic independent of the predominant first harmonic tangential force

Another rather strange observation should be mentioned A change in the Lissajoux figures corresponding to a 180° phase shift was sometimes observed without reversal of the direction of rotation, but with change of placement on the leather This observation was not sufficiently reproducible to permit a detailed study of the phenomenon However, by placing a pick up needle in a small groove on the leather and moving the electrode to and from the pick up it was observed that sudden increases in pressure seemed to affect the phase shift, pressure changes being effected by unevenness in the leather surface Reproducable observations were the 180° phase shift for the second harmonic under direction reversal of the electrode movement

Location of the sound source

The observations of phase shift led to the question whether the perceived fricative sound produced on leather was radiated from the vibrating electrode or from the leather

If the electrode was the main sound radiator, a rather thin string electrode ought to produce very little sound, being a high order sound radiator of small dimentions compared to the wavelength and therefore, a poor one

If, in addition the immediate surroundings of the electrode was provided with sound absorbing material such as a wrapping of cotton and an impervious outer cover of plastic and/or a fiber tube, the radiated fricative sound should be very weak

An experiment with such an electrode was carried out and no appreciable reduction of radiated fricative sound was observed compared to the sound from the commonly used electrode system

Therefore, it was concluded that the main sound source in the fricative effect was the semiconductor itself, i e , the skin the leather etc

(The possibility of approximately equal radiation of sound from leather and electrode might still excist, because then only a 3 dB decrease should be observed if the radiation from the electrode was excluded However with the electrodes used the conclusion that the semiconductor itself was the main sound source should be safe)

This conclusion agrees very well with the experience from the fricative electrophonic effect Electrode movement away from the ear usually produced a louder fricative sound than did movement toward the ear, especially for the first harmonic component If the sound was produced mainly in the skin close to the electrode the tangential sound vibrations should be more easily transmitted to the ear by cartilage conduction when the skin between the electrode and the ear canal was stretched by movement away from the ear

The second harmonic component in the fricative electrophonic effect was most easily perceived when the electrode was rubbed against skin over bony parts of the head Besides the possibility that the properties of the skin on such places make the production of second harmonics more predominant (Johnsen Rahbek phenomenon) it must also be considered that the normal component of the fricative sound will be more easily transmitted by bone conduction than any tangential component

The experiments on leather indeed showed that the normal component contained more second harmonics than did the tangential

5 1 7 3 Discussion (Johnsen-Rahbek versus electrical adhesion)

The sound production and sound conduction in the fricative effect are so tied together that it is very difficult to investigate them separately This makes it extremely difficult to unveil the mechanism of the fricative effect in general as well as the more specific fricative electrophonic effect

When a moving electrode is applied to leather it is difficult to differentiate between the leather acting as a fricative transducer and acting as a transmitter and/or radiating body When the fricative sound is observed to be loudest, is this due to the optimum ability of the leather to radiate sound or to its acting as a fricative transducer?

The radiation ability of leather under different wetness conditions was tested qualitatively using a bone conduction vibrator repeatedly pressed against the leather It was always applied on the same spot and the radiated sound was observed as the wetness was varied

It was obvious that increased moisture reduced the radiation of sound

On the other hand it was possible to produce audible although faint fricative sound from leather so soaked that water was visible on the surface

Thus a fricative transducing mechanism may function under extremely humid conditions (100% humidity)

A transducing mechanism like this can hardly be explained as the regular Johnsen Rahbek effect since this effect is spoiled by humid conditions In addition, the first harmonic component was not cancelled by any polarizing voltage, thus excluding the possibility that the observed first harmonic was caused by a polarizing voltage produced by the system itself, i e , by contact potentials or «static» electricity produced by the movement

Most probably two different mechanisms are involved in the fricative effect

a) The genuine Johnsen Rahbek phenomenon in which a variable electrostatic force is modulating the sliding movement of the electrode and producing a second harmonic tone, if no polarizing voltage is acting The modulating adhesive force is independent of the polarity of the electric field between conductor and semiconductor

b) An electrical adhesion phenomenon in which the adhesion force is depending upon the polarity of the field between the electrode and the semiconductor In this case the sliding movement of the electrode is modulated only once during each cycle of the applied alternating current thereby producing a first harmonic tone

The first of these two mechanisms is well known in the physical literature (Johnsen Rahbek effect) and needs no further explanation (Geiger and Scheel, 1927) The experiments earlier described demonstrated the presence of this mechanism in fricative effect under specific conditions

The second mechanism is to my knowledge not yet described in the physical literature It may therefore be desirable to report briefly some considerations and experiments which have been made in order to verify the hypothesis of the polarity dependent electrical adhesion force

5 1 7 4 Adhesion Force as a Function of Polarity and Electric Field Strength

Direct measurements of the adhesion force

In order to measure the adhesive force as a function of polarity and of voltage (and/or current) a sensitive balance (Cenco Surface Tensiometer) was used

It proved to be impossible to increase the electric field beyond a very limited value because leather and other semiconductors were too easily burned or had their properties changed at the contact area by higher current strength The more it was necessary to make the tensiometer extremely sensitive, which required very small and light electrodes Small gold-plated brass and steel balls were used in these experiments, – plated according to the method by Kohlrausch (1910) The electric current was applied to the electrode over the torsion arm through a contact system formed by a thin platinum wire immersed in a groove with mercury (Fig 15)

A series of experiments were performed with this apparatus and it is beyond the scope of this study to report in detail about these

The most important work was to measure the torsion force necessary to overcome the adhesion between a piece of leather and the electrode when various DC voltages of different polarity were applied to the electrode The result of a series of experiments are presented in a diagram in Fig 16

Although the results do not reflect a completely unequivocal conformity to a polarity law, it is evident that an electric adhesion force is present when the metal electrode is made positive and the leather negative If the polarity is reversed it is usually observed a repulsive force between the leather and the electrode, completely different to when the voltage drop over the electrode/leather is made zero

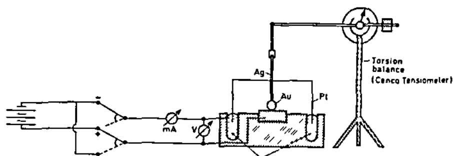
One experiment that demonstrated this convincingly was to adjust the torsion wire so that it just balanced the regular adhesion between leather and electrode when no electric field was introduced The application of 10 – 15 V positive voltage to the electrode did not call forth any visible change but it was possible to adjust the tensiometer screw to a greater pull on the electrode without eliciting a break between the leather and the electrode If 10 – 15 V negative voltage was applied to the electrode it always broke loose from the leather At the same time one could observe water moistening the electrode as if fluid was transported from within the leather towards the electrode

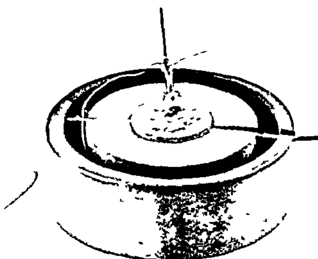
There was no such observable liquid between the electrode and the leather when the electrode was made positive and the electric adhesion force was overcome by increased torsion force from the wire

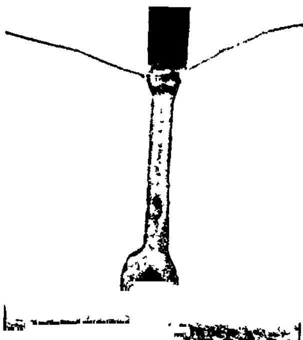
Indirect measurements of the adhesion force

Another attempt was made to obtain quantitative data for the electric adhesion force as a function of the electric field strength and polarity by observing

Fig 15 Electrical adhesion experiment
a) Schematic drawing of apparatus for measuring the adhesive force between a piece of leather and a gold plated brass electrode when various DC-voltage of different polarity was applied to the electrode







b

b) Photography of the container for Hg and leather and the cables for supplying the electric energy

c) Photography of the electrode at the moment of torsion force overcoming the adhesive force between leather and electrode

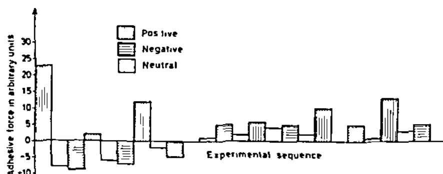


Fig. 16 Measured adhesive force with the apparatus shown in Fig 15 as a function of polarity of the electrode

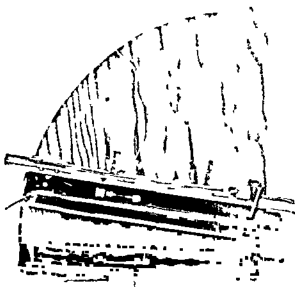


Fig 17 The two incl ned planes with the mov ng electrodes and the starter and the start ng relay etc At the bottom the V shaped inclined plane and above it the regular plane with poss bility of precise variation of its angle

the fall time of a sliding electrode (gold plated brass) on an inclined plane made of two leather stripes on metal forming a V shaped conduit and also by a single leather stripe forming the inclined plane (Fig 17) DC voltage was supplied to the moving electrode by means of a very light insulated wire and the inclined plane itself was connected to the other terminal of the DC supply Voltage and current were registered and a switch made it possible to reverse the polarity The angle of the inclined plane was usually kept between 30° and 45° The sliding electrode was at the starting point influenced by a small spring force which made start conditions identical under each experiment The electrode was started by a relay and the fall time along a distance of about 40 cm of the inclined plane was measured with an electric clock by means of one starting relay at the beginning of the measuring distance and one closing relay at the end of it

Unfortunately like in the other experiments with the balance it was not possible to carry out the investigation within a wanted voltage range because the leather was rather easily burned or definitely changed at the contact area with the moving electrode when a current of more than 50-100 mA passed through the system

The important observations may be summarized as follows An increased fall time (about 20 per cent) was measured when the sliding electrode was positive compared to zero or negative voltage If the voltage was too high (high in this connection was dependent upon the humidity conditions of the leather for instance 20-30 V for very wet leather) the electrode would not start when using a reasonable angle of the inclined plane (less than 60°

Making the electrode electrically neutral did not affect a start But when the voltage was kept unchanged and the polarity reversed the electrode started like a «shot» as if influenced by a repulsive force

Applying an alternating current of frequency in the audible range (General Radio Oscillator) did not greatly influence the fall time although it usually was increased enough to be measured The fricative effect however was nicely demonstrated As the electrode slided down the inclined plane it produced a distinct audible fricative sound

These experiments demonstrated the presence of a polarity dependent adhesion force between a metal electrode and a semiconductor like leather However the transportation of water (or liquid) within the leather when applying an electric field between the leather and the electrode probably made the properties of the leather continuously changing thereby making it extremely difficult quantitatively to vary one parameter at a time without influencing others

Although a lot of time was spent doing a series of experiments with various semiconductor systems the fricative transducing mechanism is not considered to be completely unveiled quantitatively In a number of different experiments using various types of proteins as semiconductors cataphoresis and electro-endosmotic transport seemed to indicate that the molecular structures of the semiconductor just underneath the metal electrode may rapidly be changed depending upon the polarity Such changes may be caused by water molecules transported or oriented differently under the influence of varying electric fields

If this assumption is correct a complete solution of the fricative transducing mechanism probably is connected to the very problem of friction not yet solved in detail

A further study should probably consider the following two possibilities

I) An electrostatic force such as described in the Johnsen-Rahbek phenomenon, modified and even destroyed by electroendosmotic fluid each time the polarity causes to flow to the electrode

II) Molecular forces between semiconductor and conductor The difference of the two materials as to the mobility of electrones and holes may cause some type of electric double layer (dipole-effect) or structural patterns in which there occurs a sharing of molecular elements In this way both the charging of the semiconductor and the distance between the charges on the conductor, respectively the semiconductor, may be polarity dependent Therefore, the adhesive force between the conductor and semiconductor varies with polarity and with type of semi conductor and even with different places on a non homogeneous semiconductor

Possibly a more general explanation of both the Johnsen Rahbek phenomenon and the fricative effect's first harmonic sound may appear to exist



Fig 18 Large area electrode on mandibular joint A fiber tube electrically insulates the holder from the electrode itself

5.2. Large area electrode.

1. Apparatus and procedure

As already mentioned in the chapter of the fricative effect, a sound was sometimes produced without moving the electrode, when the area of the electrode was rather large and the applied voltage rather high

When the contact area of the electrode increased beyond about 150 mm^2, one might experience the electrophonic perception of the large-area electrode, i e , sound production with a stationary electrode

This electrode system was in principle the same as that used by the «Radio enthusiasts» (1925) in «Funkscherz», Kosack (1926), Gernsback (1926) and by Jellinek and Scheiber (1930), Perwitschky (1930) and Kahler and Ruf (1931)

With the indifferent electrode strapped to the forearm as before, a large area electrode (a brass cylinder, 18,6 mm in diameter) was placed on the skin over the mandibular joint the place of contact first washed with ether (Fig 18) Dry skin on any part of the head also produced a sound effect

5 2 2 Auditory response

When a sinusoidal signal of rather low voltage was applied, a sound was perceived by the subject Increasing the voltage, other persons might hear the sound as it was radiated from the place were the electrode was located

With the voltage kept constant at such a value that the tone was heard at the start of the experiment, the current immediately started to increase and the loudness of the perceived tone decreased to inaudibility Fig 19 shows a typical record of the current as a function of time for 8 V between the electrodes Somewhere between 0 2 and 0 3 mA the sound became inaudible

It is obvious that a reliable description of the auditory response was very difficult in such a transient system A thin dielectric e g a sheat of cellophane between the active electrode and the skin, was to a large extent able to stabilize the sound effect

Perspiration caused a very thin paper to become damp and the transient character of the sound reappeared

It was however, possible in the earlier described way in chapter 4 (General Methods) to analyze the

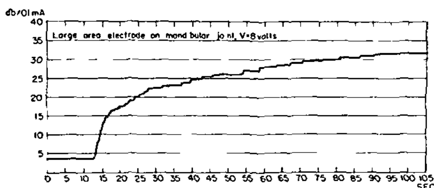


Fig 19 Increase of current as a function of time For this particular placement of the active electrode the applied voltage was 8 volts Curves of similar shape were obtained for various electrode placements and for various values of the AC potential although a faster increase usually resulted from a higher potential

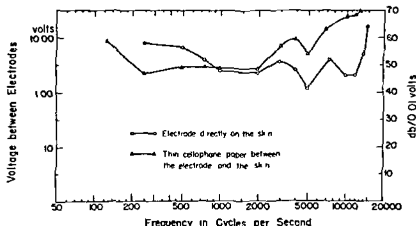


Fig 20 Threshold curve for a large area electrode (*diameter 17 mm*) *on the mandibular* joint The subject s ears were plugged during the experiment

composition of the perceived tone It contained both first and second harmonics Below 2000 Hz the second harmonic was dominant while the first harmonic was more pronounced above 4000 Hz As shown in Fig 20 it was possible to hear tones when using signal frequencies at the upper limit of hearing

5 2 3 Threshold

The threshold values were obtained by having the subject barely to lift the electrode from the skin and then let it return to its original position The voltage was adjusted by means of a 1-dB step attenuator so that the fluctuating sound could just be heard when it was at its loudest Fig 20 shows the results for one subject with plugged ears It was possible to hear lower signal frequencies with a thin dielectric placed between the skin and the electrode than when the electrode was in direct contact with the skin Even with a dielectric there was no perception of sound at 125 Hz but rather a tactile sensation a feeling of vibration of the head or inside the head

5 2 4 Loudness of the produced sound

Because of the relatively high threshold voltage it was not possible to obtain high intensities with a large area electrode Loudness matching of an electrophonic to an acoustic sound showed that the intensity of the electrophonic sound increased with the square of the applied voltage The highest sensation level obtained for the electrode used in these experiments diameter 18 6 mm was approximately 40 dB

5 2 5 Effect of Polarizing Voltage

Assuming that the transducing mechanism is governed by a square law the introduction of a polarizing voltage superimposed upon the sinusoidal voltage should result in certain sound products which analyzed should prove the existence of the assumed square law

$$L = kV^2 = k(V_P + V_A \sin\omega t) = k(V_P + 2V_P V_A \sin\omega t + V_A \sin^2\omega t) \quad k(V_P^2 + 2V_P V_A \sin\omega t + \tfrac{1}{2} V_A (1 \cos 2\omega t))$$

Polarizing voltage = V_P

Sinusoidal voltage = $V_A \sin\omega t$

Transducer coefficient = k

According to the equation the sound products should consist of a mixture of first and second harmonic sound The first harmonic should be proportional to the product of the polarizing voltage and the amplitude of the sinusoidal voltage The second harmonic sound should be independent of the polarizing voltage only proportional to the square of the amplitude of the sinusoidal voltage

In broad outline the experimental results with good approximations confirmed the validity of the square law response

The small discrepancies observed may by related to the following two features

I The body presents a rectifying (polarizing) effect to an alternating current This fact explains why a 1000 Hz electrical signal was heard as a complex tone containing both 1000 Hz and 2000 Hz If a variable DC potential was supplied to the circuit from an external source the polarizing voltage from the body could be compensated so that only the second harmonic was heard The adjustment was very sharp For one subject the superimposed DC potential at 1000 Hz was 0 25 V for a signal of 6 volts RMS in order to make the first harmonic electrophonic sound inaudible The ability of a very small DC potential to elicit the first harmonic is reflected in the equation for the square law response
Two times the product of the polarizing and the alternating potential ($2V_PV_A$) is responsible for the intensity of the first harmonic tone Therefore only a very small rectifying effect or a contact potential may be able to call forth a first harmonic tone in spite of the acting square law transducing mechanism

II A negative potential applied to the active electrode had a disturbing side effect It caused an annoying pain and tended to make the sound production more transient This is probably correlated with the substantial reduction of the impedance of the skin which was so strikingly observed when the system was polarized with a negative potential By reversing the polarity the original impedance was largely restored This phenomenon indicates an electro-endosmotic process in which the positively charged water is transported to the negative electrode For clarity it should be pointed out that the pain was usually not caused by the polarizing voltage alone but by the DC voltage together with the sinusoidal current Neither was the pain due only to the higher current that was induced by the diminished impedance because much less pain resulted when the same voltage or current was superimposed by a positive polarizing potential (It usually took a little time until the impedance was restored when reversing the polarity)
Beside the pain effect the negative polarizing voltage may cause a disturbing effect upon the transducing mechanism itself because of the increased humidity at the electrode

5 2 6 Location of the Transducer

The results from experiments with the large area electrode show that the transducing mechanism in this electrode system can not be located in the cochlea

The transient effect the sound radiation from the skin and the square law response indicate an electrostatic transducer located at the place of the contact between the electrode and the skin

5 2 7 The transducing mechanism

A detailed explanation of the transducing mechanism in the large area electrode system should include a description of what is vibrating the metal electrode or the dielectric or both and also the relation between the mechanical vibrations and the applied electrical signal (alternating voltage)

Chocholle (1948) in one of his later publications regarding the electrophonic effect states that the large area electrode electrophonic effect is analogous to the vibrations of a dielectric material

placed between two condenser plates which are connected to each pole of an electrical oscillator He proposes that the skin itself is the dielectric material which expands and contracts in rythm with its surface charges induced by the active electrode In other words he ascribes the vibrations to the dielectric material i e the skin

The other possibility is that the electrode itself is the vibrating part

The second possibility seemed to be the most probably one since the most likely transducer in this electrode system was an electrostatic one of the condenser type It works according to a square law the mechanical force (F) acting between the plates being expressed in the following equation

$$F = \frac{\varepsilon V S}{8 d^2}$$

ε is the dieletric coefficient
V is the applied voltage S the electrode surface area d the distance between the two acting plates

When applying a sinusoidal voltage to the plates the sound intensity (L) produced by such a transducer may be expressed as a function of the applied voltage (V) and the electrode surface (S) when neglecting the small variations in distance (d)
$L = k V^2 S$

If it is assumed that the electrode and some underlying part of the skin beneath the electrode form the two condenser plates with air and layers of the skin constituting the dielectric between the plates the vibrations of the electrode should be expected to be transmitted as bone conduction to the inner ear

In an earlier work (Flottorp 1953) it was experimentally shown that the equation for the electrostatic mechanical force was fulfilled This does not mean that the electrode necessarily is the vibrating part or the only one causing the sound production

If the electrode was the vibrating body acting on the skin and bone underneath then the mass of the electrode should determine the vibration amplitude the force being described by the equation An applied voltage should in the electrostatic transducer system be transformed into a specific force acting upon the electrode according to its surface area (and distance between the acting plates which can not be deliberately altered) Depending upon the mass of the electrode the vibration amplitude should vary resulting in various intensity of the electrophonic sound Increasing the mass of the electrode should decrease the vibration amplitude i e the sound intensity and vice versa In order to test this the mass was increased in weight 100 fold over the standard electrode then it was reduced to one hundredth of the usual weight In neither instance the threshold voltage for hearing the electrophonic sound changed appreciably

This seems to indicate that the metal electrode is not the vibrating part solely responsible for the electrophonic sound in the large area electrode It rather points to the skin as the vibrating dielectric

Vibrations of the skin must be transmitted through the bone to the inner ear to produce a hearing sensation In addition part of the vibration energy may be transported to the ear canal via cartilage conduction depending upon the placement of the electrode The mechanical impedance of the skin is quite different from that of the bone and also different from the impedance of the air With a very light electrode mounted to the skin the impedance match is assumed to be better in the direction away from than towards the bone and less energy is expected to be transmitted to the inner ear

If the large area electrode has some mass it should constitute a higher impedance improving the realtive impedance match to the bone and more vibration energy should be reflected from the electrode to the underlying structures and subsequently to the inner ear

This was checked using a metal leaf 1/1000 inch thick as the large area electrode The threshold with this electrode appeared to be approximately 6 dB higher than with the other electrodes of appreciable mass

From these two experiments it may be concluded that the electrophonic hearing in the large area electrode is not only due to vibrations of the electrode but rather to vibrations of the dielectric material i e the skin itself

In order to study these vibrations and to investigate the hypothesis of Chocholle (1948) a particular condenser system was made with the one used by Chocholle as model

5 2 7 1 *Experiments with Analogue Systems*

Two very heavy condenser plates of brass were made as cylinders diameter 10 16 cm length 11 2 cm each of 7 6 kg weight (heavy because they

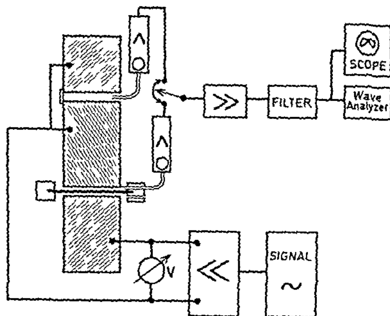


Fig 21 Block diagram of electrostatic transducersystem of heavy condenser plates (7,6 kg each) and the control by means of an extra gap at the rear end of one of the plates

should vibrate as little as possible). (Fig 21 and 22)

The calculated electrostatic force between the plates for a distance d=1 mm between the condenser plates and the space between the plates completely filled with a dielectric of $\epsilon = 3.3$ and 100 V applied to the plates, was 100 dynes

The maximum vibration amplitude of one of the plates in response to an alternating voltage of 100 V at 1000 Hz should be approximately $5 \cdot 10^{-11}$ cm This is far less than the diameter of the hydrogen molecule and also less than the amplitude of the eardrum at threshold in the frequency range for which the ear is most sensitive (Wilska 1935) Theoretically, the vibration of the plates in this condenser system should be of no significance

The following experimental control was carried out in order to confirm the calculations

The two heavy condensor plates were mounted with parallel end surfaces in a distance of 1 mm by means of a lathe When supplying a signal of 1000 Hz, 100 V to the plates no sound was registered from the gap neither objectively with a sensitive microphone, nor subjectively using a stethoscope

Placing a sheet of paper between the two plates produced an audible sound of 25 30 dB SL

The increased electrostatic force between the condenser plates caused by the paper having a higher dielectric coefficient than air was calculated to amount to 12 dB It was concluded therefore that the observed sound must originate from vibrations of the paper An extra control of this was made based upon the following reasoning If the radiated sound was caused by vibrations of the plates, the establishment of a similar gap at the rear end of one of the plates by means of another plate electrically connected to the one in question, ought to produce radiation of sound from the new gap The introduced

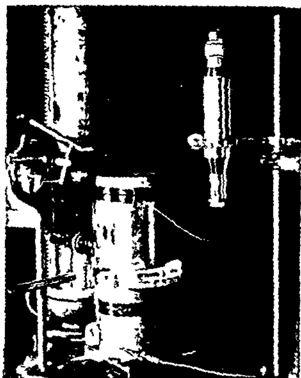


Fig 22 Photography of the electrostatic transducer system with probe microphone

extra plate did not cause any sound to be radiated from the new gap either (See Fig 21)

It should therefore be safe to conclude that in the actual electrostatic system the transducing mechanism was bound to the dielectric material placed between the two condenser plates

The next question was now How does the dielectric material vibrate causing the radiation of sound?

It seemed to be six possible mechanisms for a sound production by a dielectric material in an alternating electric field between two condenser plates

I Chocholle s theory which in short states that the opposing charges on each side of the dielectric sheet will attract each other and therefore compress the dielectric material each time it is charged This would occur two times per cycle of the alternating voltage supplied to the condenser plates thus producing a second harmonic sound

II The effect of Maxwell s stress tensor i e an electrostriction phenomenon The electrostrictive force (F_n) is always a tension away from the material of higher dielectric coefficient (ε) to that of lower (ε_1)

$$F_n = \frac{\varepsilon_2 - \varepsilon_1}{8} E$$

$$E = \text{Electric field} - \frac{V_A \quad \sin\omega t}{d}$$

(Many textbooks describe Maxwell s stress tensor in the present work the description by Hylleràs 1950 has been used)

The physical explanation of the equation is that the dielectric sheet suffers an extention in the direction of the applied alternating field not a compressive force as in Chocholle s hypothesis A second harmonic tone is the result also here the force governed by a square law

III The piezoelectric effect which is much the same as the electrostriction phenomenon except that the dielectric extends and contracts depending upon the polarity of the field thus producing a first harmonic sound

(It seemed rather improbable that the various types of dielectric material used in the present experiment should all have piezoelectric properties while hitherto mainly certain crystals have been found to have them)

IV The dielectric material being charged in some way causing the sheet to vibrate under influence of the alternating field

A mechanical force proportional to the charge and to the applied voltage should act upon the dielectric material producing vibrations of the same frequency as the alternating voltage to the plates i e a first harmonic sound

V Because the dielectric material could hardly be completely parallel and symmetrical to the condesor plates it should be exposed to assymetrical alternating forces causing a second harmonic sound

VI Convexness in the dielectric material might be increased under influence of the Maxwell stress tensor and give rise to a second harmonic sound

A systematic study of these six possibilities was carried out by analyzing the produced sound together with measuring phase relations on both sides of the dielectric material The results showed that the sound was composed of both first and second harmonic with first harmonic as the predominating component When using a cellophane paper of about 1/10 mm thickness and a distance of approximately d = 1 mm between the plates and applying V = 100 V to the system the sound was easily heard in the experimenting room for frequencies up to 15 000 Hz In the lower frequency range in which the ear is less sensitive the sound was just audible

In order to study objectively the sound production a small probe tube was mounted to a Western Electric 640 AA condenser microphone and the phase of the sound wave in comparison to the oscillator output was measured at various points on each side of the dielectric material A phase difference of 180° was always observed in corresponding points on each side of the dielectric

The microphone demonstrated mainly a first harmonic component while the use of a stethoscope

and the method of best beats showed the presence of a second harmonic tone also

The results seem to exclude Chocholle's theory, and also the piezoelectric effect, since both of these hypothesis presume a pulsation of the dielectric material causing the sound pressure to be in phase on each side of the dielectric Chocholle's theory presupposes a second harmonic sound which was found to be very weak, whereas the first harmonic was the most predominant component The analogue experiment thus showed results divergent from the large area electrode data from humans

It was, however, considered of interest to complete the analogue experiments, because they might elucidate some of the more vague phenomena in the large area electrode system

The most probable transducing mechanism in the analogue system

The predominant first harmonic component seemed to indicate that vibrations of the charged dielectric might be a probable explanation of the tone production The equation governing such a transducing mechanism, expressed as the acting force F on the dielectric, may be written as follows

$$F = k\,Q\,\frac{V_A \sin\omega t}{d}$$

Here k is a constant depending upon the units, Q is the electric charge on the dielectric,

$\frac{V_A \sin\omega t}{d}$ is the electric field expressed by the applied signal to the condenser system and the distance between the condenser plates (d)

The equation contains three independent variables, which could be varied separately, making it possible to check the validity of the hypothesis The mechanical force F is related to the sound intensity I, and the microphone output voltage (U_m) to the sound intensity (I) according to the same law Therefore, the output voltage from the microphone should be proportional to the applied voltage to the plate, keeping the charge (Q) and the distance (d) constant

In the control experiments it was necessary to secure that the condenser plates and the dielectric material were exactly parallel This was obtained by mounting different types of dielectric sheets into circular frames, so that no bulges appeared on the dielectric surface (Fig 21 and 22) The diameter of the frames were slightly larger than the diameter of the condenser plates The dielectric material was then placed in complete contact with one of the condenser plates, the other plate successively moved to make complete contact on the other side, and the frame with a dielectric material was finally fixed securely in this position Then the two plates were separated to allow a suitable gap (d)

The three part in the condenser system were in this way always parallel

When the applied alternating voltage to the plates was varied, the microphone output was observed to vary in direct proportion to the applied voltage confirming that part of the equation

According to the equation the microphone output voltage should vary inversely proportional to the distance (d) i e the product of microphone voltage (U_m) and plate distance (d) be constant

A series of measurements gave the following results for this product

Distance d (cm)	Microphone output U_m (volts)	U_m d
0 15	0 5	7 5 10^{-2}
0 40	0 35	14 0 10^{-2}
0 55	0 30	16 5 10^{-2}
0 65	0 21	13 7 10^{-2}
0 85	0 16	13 6 10^{-2}
1 10	0 13	14 3 10^{-2}
1 50	0 095	14 2 10^{-2}
	Average of the last 6 values	14 4 10^{-2}

With the exception of the very short distance (d=0 15 cm) – for which the percentage uncertainty of the distance measurements is by far the greatest – the product U_m d is constant within reasonable limits (+15 per cent – 5 5 per cent) thus confirming that part of the equation

The most difficult task in checking the validity of the equation was with respect to variations of the charge (Q) which is difficult to measure

In lack of suitable experiments and method to make precise measurements of the charge of the dielectric material a more qualitative check was made based upon the following reasoning

If the charge was removed from the dielectric, the

sound should disappear (i e the first harmonic component) Recharging the dielectric should then reproduce the sound

Since it seemed very difficult to have a dielectric material placed between the two condenser plates without charging it the problem was to remove the charges Several means were tried such as ionization of the air between the dielectric and the plates by means of ultraviolet light from a mercury lamp *and using thorium grains* ($ThCl_4$) *placed on both* sides of the dielectric material and also experimenting with air saturated with water The final solution was an antistatic spray designated for removing electric charges from films

The antistatic spray was used on various types of dielectric materials all of which were mounted parallel to the condenser plates in the manner described Briefly the results may be summarized as follows

A douche of antistatic spray might result in a temporary further charge of the dielectric material eliciting an increased sound production Then the decharging effect came into force reducing the sound intensity to inaudibility with respect to the first harmonic sound a very faint second harmonic sound remained

Recharging the dielectric material for instance by rubbing it with cotton produced a first harmonic sound which disappeared after a few minutes The effect of the antistatic spray lasted for a day or two

It was considered necessary to study the sound production from dielectric material having properties more related to the skin than the hitherto described sheets of plastic or paper The human skin has different layers of different electric quality some parts acting almost as an insulator other parts containing blood vessels etc more like an electrolytic conductor

In this respect chamois and soaked filter paper between cellophane sheets were considered to have some resemblance to the human skin Experiments with these dieletric materials shall be reported in more details

Dry chamois as dielectric material caused only a doubtful faint sound production if any Rubbing the leather with cotton (charging it) called forth a distinct first harmonic sound however no second harmonic as measured by best beats Use of the antistatic spray removed the charge and the sound production gradually faded away

Moistening the chamois with water produced a sound containing both first and second harmonic tones The second harmonic tone lasted longest as the leather dried

A filter paper soaked in water and mounted between two cellophane sheets (5/1000) in a polystyrene frame was kept in water for three days to ensure elimination of all air bubbles before the circular frame was screwed tightly together and placed between the two condenser plates in the described manner There was no traceable first harmonic sound but a rather faint second harmonic tone when applying a 1000 Hz signal of 100 V to the plates

Increasing the voltage to 150 V increased the sound production and made it possible to examine the phase relationship

A phase difference of 180° was always observed in corresponding points on each side of the dielectric material If the dielectric material was brought in contact with one of the condenser plates the sound intensity increased markedly Especially favourable in this respect was a slanting position of the dielectric relative to the plates In this position the dielectric material had practically free mobility in both directions On the «free» side of the dielectric material the sound intensity was still quite high as measured with the probe tube of the condensor microphone It was also demonstrated that the sound originated from vibrations of the dielectric material

The last step in gradually changing the dielectric into more and more conducting material was the use of a sheet of copper 5/1000 thick mounted in the frame and placed between the two condensor plates as in the other experiments with dielectric sheets When a sinusoidal signal was applied to the plates a second harmonic tone was produced If the sheet was brought into contact with one of the condenser plates the sound intensity increased greatly as a result of the sheet being formed and now radiating a predominanting first harmonic sound

It was also possible to produce a first harmonic sound by charging the sheet be means of electrostatic induction touching it on one side with a grounded wire in a proper phase of the sinusoidal cycle The use of anti static spray also temporarily produced a first harmonic sound indicating that the copper had been charged by the douche

5 2 7 2 *Discussion and Conclusion*

Discussion of the experimental results

The experimental results indicated quite clearly that the first harmonic sound was produced by the vibrations of a charged dielectric (or charged conductor) in a sinusoidal electric field With good approximation the acting force might be expressed by the equation (see page 38)

$$F = k\,Q\;\frac{V_A\;\sin\omega t}{d}$$

In the large area electrode system the first harmonic sound was very weak and seemed to be caused by the polarizing voltage in the acting square law transducing mechanism The experiment with a counteracting depolarizing voltage seemed to indicate such a mechanism

In addition to this it may however be that the skin under the electrode under specific circumstances is charged and vibrates between »the condenser plates» producing a first harmonic sound (of low intensity)

The mechanism for the production of the second harmonic sound in these model experiments and therefore also in the large area electrode may probably be explained in two or three different ways

The explanation must suit the experimental finding that the dielectric vibrates as a whole body upper and lower surface being in phase – because the 180° phase difference was observed in the air on each side of the dielectric

This excludes as already mentioned Chocholle s theory and also the electrostriction phenomenon according to the Maxwell stress tensor

In the physical literature the electrostriction phenomenon is usually considered to be of very little practical importance the volume displacement being so extremely small and is therefore considered to have little bearing upon the large area electrode The remaining possibilities are thus the mechanical forces caused by asymmetrical location of the dielectric in the electric field and the uneveness and possible convexities of the surface of the dielectric again causing asymmetrical position and the same type of mechanical forces Such forces should set up vibrations of the dielectric material as a whole satisfying the requirements of the phase observations

The other experimental observation with bearing upon the explanation of the second harmonic sound production was the effect of moisture and water added to the dielectric material It may be that the water had some influence upon the mechanical structure making the surface somewhat more inhomogeneous thereby increasing possible convexities Such an explanation is in line with the assumed transducing mechanism however not so easily verified

It may just as well be some new physical properties given to the dielectric making it vibrate according to a square law in the alternating electric field

The final conclusion to be drawn from the model experiments was that vibrations of the dielectric according to a square law seemed to be of rather little importance being a very little efficient transducing mechanism The first harmonic sound caused by the charging of the dielectric represented a much more efficient mechanism

It therefore seems necessary to explain the large area electrophonic effect by means of additional vibrations of some condenser plates according to the physical principle for such electrostatic forces

The transient effect in this electrode system and the restoring of the sound production introducing a thin dielectric sheet between the skin and the electrode strongly point to the very thin surface area of the skin under the electrode to be the important place of the transducing mechanism If this part of the skin in dry condition has outstanding insulation properties then the electrostatic forces between the electrode and the conducting layer just underneath the insulating layer of the skin may be very high in spite of rather low voltage difference

The vibration of the conducting layer which may include even the underlying bone will then be transmitted to the inner ear as bone conduction Such a transmission will take place independant of the mass of the active electrode as long as it has sufficient mass to represent some higher impedance than the skin itself in the direction of the surrounding air As shown experimentally an extremely thin electrode had influence upon the electrophonic threshold probably because of less efficient transmission to the inner ear (i e more vibration energy transmitted to the air)

In addition to this effect a vibration of the dielectric itself i e the skin having insulation properties together with a »sheet» with some

«moisture» in it may vibrate under influence of assymetrical electrostatic forces These vibrations will be in phase with those of the condenser plates but probably of much less amplitude

Conclusion with respect to the electrophonic effect

The perceived electrophonic sound when using a dry electrode applied to the skin somewhere on the head (and even to a metal filling in a tooth) is caused by mechanical vibrations transmitted to the inner ear mainly as boneconducted sound maybe also as cartilage conducted and air conducted sound from the area underneath the electrode Here the transducing mechanism is located being of electrostatic origin following a square law thus producing a second harmonic tone In addition a first harmonic tone may also be perceived produced at the same place either because of a polarizing effect on the electrostatic transducer or by a dielectric sheet (i e part of the skin) being charged by contact potentials or rectifying effect in the body No experimental fact supports a hypothesis that the applied voltage (current) reaches the inner ear and there give rise to some action responsible for the sound perception

5 3 The Brenner method

5.3 1 Apparatus and Procedure

The most common means of electrophonic stimulation has consisted of an active electrode emerged in a salt solution (Ringer solution physiological NaCl solution) in the external auditory meatus The passive electrode was usually strapped to the fore arm a piece of cotton wool moistened in the salt solution providing good contact with the skin

Although this electrode system was used long before Brenner (1868) (f i by Weber 1846) it is usually referred to as Brenner s method

The qualitative and quantitative properties of electrophonic perception with this electrode system were thoroughly investigated by Stevens (1937) and Stevens and Jones (1939) Their circuits and methods have served as a model for the investigations reported here A block diagram of the apparatus is presented in Fig 2

The subject was placed horizontally with his head comfortably resting on a pillow and the ear under test facing upwards An earphone of an audiometer was placed on the ear resting on the pillow and a bone vibrator was connected to the forehead (or sometimes to the mastoid bone) By means of a movable arm connected to a special headband the electrode could be located in the meatus which was filled with the salt solution (Fig 23 and 24)

The subject compared the perceived electrophonic sound with the airconducted sound presented to his other ear or he reported about beats with the bone conducted sound according to the earlier described procedure in chapter 4 General Methods

Normal hearing persons and also a few persons with pathological middle ears served as subjects

5 3 2 Auditory Response

When a sinusoidal current of adequate frequency and intensity was passed through the system the subject heard a mixture of first and second harmonic tones This mixture was a function of the frequency it varied somewhat from person to person and probably also with the time meatus had been filled with the electrolyte It was therefore difficult to describe the auditory response unequivocally as a function of frequency and voltage/current of the stimulus

The experience from this work fits well with reports of different experimentators in electrophonic hearing using the Brenner method Some researchers like Kellaway (1946) reported the perceived sound to have a pitch corresponding to the applied signal frequency (i e a first harmonic tone) while others like Stevens (1937) reported a mixture of first and second harmonic tones and others again mainly second harmonic

Below 150 Hz listeners in this work usually did not hear a tone but might feel some vibration Some of the subjects without eardrum reported the sensation of noise a roaring sound sometimes together with a tone sometimes only the noise

5 3 3 Threshold

According to earlier practice (Stevens 1937 Stevens and Jones 1939) threshold for a tonal sensation without differentiating between a first and second harmonic sound was determined using a 1 dB step attenuator to vary the voltage between the electrodes Threshold curves for some subjects with normal middle ear and without eardrum are presented in Fig 25

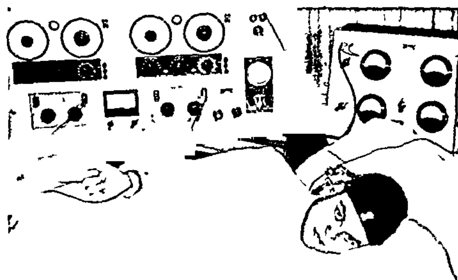


Fig 23 Subject in test situation in the Brenner method

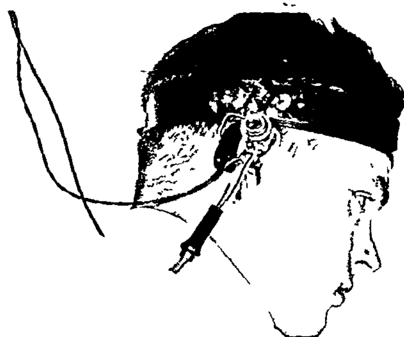


Fig 24 Head of the subject with bone conductor and headband with moveable arm holding the electrode in the ear canal

In pathological ears no threshold could be obtained in frequency regions where the bone conduction threshold was poor, i e indicating hearing loss greater than about 40 dB

5.3.4 Loudness of the Produced Sound

Because the threshold of shock is rather close to the threshold of hearing it is difficult to perform a loudness comparison between an airborn sound and the electrophonic sound for intensities above a sensation level of approximately 30 dB However, it was possible to show that the sound intensity increased with the square of the voltage between electrodes in good agreement with the result reported by Arapova et al (1937) shown in Fig 7

5.3.5 Effect of Polarizing Voltage

By adjusting the voltage of an externally applied

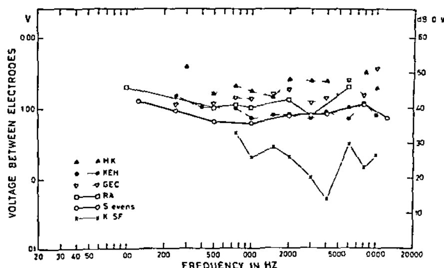


Fig 25 Threshold curves for electrophonic hearing Brenner method for ears with normal middle ear (GEC and KEH) and for ears without ear drum (radically operated ears RA and K SF suppurating ear with large perforation H K) For comparison the average threshold curve for the Brenner method as obtained by Stevens is also presented

polarizing potential it seemed possible to eliminate the first harmonic (compensating the polarizing and/or rectifying effect of the body) However a very careful investigation among trained observers indicated that a very faint first harmonic tone remained and could not be eliminated by any polarizing voltage These findings were to a certain degree dependent upon the chosen frequencies

The predominant result of a polarizing potential was however the striking experience of loudness increase of the first harmonic component when increasing the polarizing voltage either in negative or positive direction

Also the findings reported by Stevens and Jones (1939) «A given change in attenuation has a much more impressive effect on the loudness of the tone than an equivalent change has on the loudness of the fundamental when the ear is polarized» – were *confirmed*

The effect of the polarization upon the electrophonic sound indicated that a square law governed the electrophonic effect in the Brenner method This has been thoroughly described in the work of Stevens and Jones (1939) and is also explained mathematically in the present work in chapter 5 2 5 on page 34

5 3 6 Location of the Transducer

5 3 6 1 Theoretical and Experimental Considerations about the Role of the Electrode

Based upon the square law response found for the Brenner method in normal ears Stevens and Jones (1939) Jones et al (1940) ascribed the transducing mechanism to a condenser formed by the eardrum and the promontory Because they thought it too dangerous to fill a salt solution in an operated ear lacking eardrum they changed the electrode system when examining such ears without being aware of the consequences such a change might have upon the perceived sound They found a different response to the applied alternating current in such pathological ears and considered the eardrum and middle ear to be important structures in the tranducing mechanism governed by the square law

A control of the calculation used by Stevens and Jones (1939) in their attempt to show the probability of a condenser formed by the eardrum and the promontorium revealed that at least 20 dB lower pressure was produced by the presupposed condenser system than indicated by their calculations This result makes it very difficult to assume that such a condenser system might at all be able to produce an audible sound even when working without any electrical leakage between the two condenser «plates» – moreover a rather unrealistic assumption for a condenser system formed by the actual structures

Russian scientists continued to use the Brenner system when they examined persons lacking ear drum (Arapova and Gersuni 1938 and others) They reported no difference in the tonal qualities perceived by people with and without eardrum (Andrejev Gersuni and Volokov 1935 Andrejev Bronstein and Gersuni 1937)

In the present study 9 persons without eardrum showing «normal» bone conduction threshold were examined with the Brenner method

Apart from side effects, such as the perception of noise for certain frequencies, no difference was found in the perceived electrophonic sound as to pitch and loudness and complexity in ears lacking eardrum compared with normal ears Therefore, the square law response does not seem to depend upon the presence of an eardrum

In order to verify this some slight alterations of the Brenner method on normal ears were studied trying to keep the eardrum outside the circuit by not allowing it to make contact with the salt solution In one variation the salt solution was in a glass tube the far end of which made contact with a rather small spot on the meatus wall close to the opening of the ear canal In another the head of the subject was placed on its side with the ear to be examined down wards, and a glass tube ending in an earplug was fitted to the ear canal The salt solution was applied so that it just made contact with the skin of the lateral part of the meatus and did not touch the eardrum In both instances when an alternating current was applied to an electrode emerged in the solution in the tube, and an indifferent electrode was placed anywhere on the body the electrophonic perception had the same quality as in the ordinary Brenner method

The eardrum was in another experiment kept definitely outside the acting electric field by having both electrodes emerged in the salt solution in one meatus According to the definition such a system does not give a true electrophonic perception because the current does not pass through the head but only through the salt solution in the ear canal

In this experiment two enameled copperwires with conductive tips about 5 mm apart in the longitudinal direction were inserted about 15 mm into the meatus without touching the wall The obtained threshold curves for this system were similar in shape to those obtained with the Brenner method on the same ears An example is shown in Fig 26 The curves are almost parallel, the Brenner method being the most sensitive by approximately 7 dB This difference might be larger or smaller depending upon the position of the two electrodes in the meatus and their relation to each other

So far the results of minor variations of the electrode system in the Brenner method seem to exclude the theory of a transducer formed by a possible condenser system in the middle ear Even more obvious should be the exclusion of the inner ear as the location of the transducer The experimental results point to the liquid itself with its electrode as the most probable place

5 3 6 2 Auscultation of Eelectrophonic Sound

In order to prove the conclusion in the preceding chapter two sets of listening experiments were carried out

In the first a stethoscope was connected to the outer ear of a rat, which under barbiturate anesthesia had the meatus filled wich salt solution and an electrode placed in it in the regular manner of the Brenner system When an electric current was passed through the ear of the animal a sound could be picked up from the meatus, whether the animal was alive or dead and whether the eardrum was present or not

In the other experiment one ear of a subject A was in the electrophonic circuit and another subject B had one ear acoustically coupled to subject A s ear containing the salt solution and the electrode The

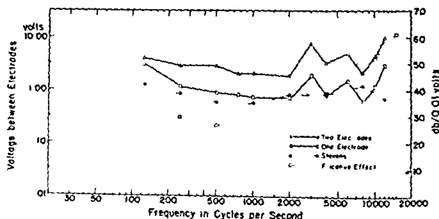


Fig 26 Threshold curves for electrophonic hearing (1) modified Brenner method with both electrodes in the salt solution in the meatus (2) genuine Brenner method with one electrode in the salt solution and for the fricative effect For comparison the average threshold curve for the Brenner method obtained by Stevens is also presented

coupling was established by means of a T shaped glass tube filled with salt solution and the copper wire with the electrode going through the stem of the T out in the meatus of subject A The «arms» of the T tube had perforated eardefenders at the tips in order to make a leakproof fit to the ear canal and at the same time conduct possible sound waves through the liquid It was difficult to obtain a leakfree connection and to get all airbubbles out of the system The T tube was chosen to be rather short and therefore the two subjects had to be placed uncomfortably with small possibilities for adjusting their relative position When passing a current of sufficient strength through the ear of subject A he heard the usual electrophonic sound which the other subject B was able to observe too

The final conclusion from these experiments was that the transducing mechanism in the Brenner method was not located in the cochlea nor in the middle ear but in the liquid with its electrodes emerged in it

5.3 7 Transducing Mechanism

5 3 7 1 Experimentation with Various Electrolytes
Apparatus and Methods

The result of the listening experiments with human subjects and with animals encouraged a separate study of sound production by passing an alternating current through an electrolyte

In some preliminary experiments the cavity of approximately the same size and shape as the ear canal was used made in a brass cylinder and filled with salt solution When passing a sinusoidal current through it it was possible to hear a second harmonic tone when acoustically connecting the ear to the cavity by means of a stethoscope with airtight fit over the surface of the liquid The sound intensity was rather low and it was therefore necessary to avoid acoustic leakages in the listening tube

The electrical circuits had also to be specially designed because any resistance of appreciable value in the circuit would consume the total voltage drop Such a mismatch may be the reason why the Russian scientists failed to make their subjects hear when the current was passed through a salt solution by means of two electrodes in one meatus (Gersuni and Volokhov 1937)

A T shaped glasstube appeared to be a practical vessel containing the electrolyte and the electrodes allowing visual control of any changes during the passage of a sinusoidal current through the liquid

The most direct way to pick up the sound seemed to be by means of a hydrophone emerged in the solution Hydrophones are however very sensitive to stray fields and almost impossible to shield from the electric field in the liquid In addition it appeared to be rather difficult to obtain a hydrophone of sufficient sensitivity and of small dimensions to suit the purpose of the planned experiments With the help of the Norwegian Defense Research Esta blishment Division for Underwater Warfare a hydrophone of usable size and sensitivity was provided

The difficulties of the stray field can be avoided using a condenser michrophone in a small airtight enclosure in the stem of a T shaped tube placed above the surface of the electrolyte

In spite of reduced sensitivity of this system compared with the hydrophone it seemed to be advantageous because it permitted to analyze both the first and second harmonic tone With the hydrophone only the second harmonic sound could be studied because of the stray field

Experiments were planned to be carried out with the use of a sensitive condenser microphone i e a Bruel & Kjær 1 microphone in a system which permitted the use of a hydrophone as well

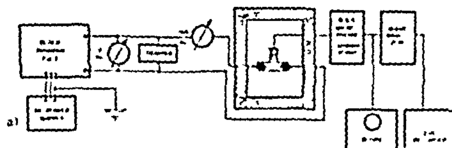
Two sizes of T shaped glasstubes were made one smaller size designed only for the use of the condensor microphone and one bigger size de signed for the use of either the condensor micro-phone or a hydrophone or both Specially designed coupling arrangements in teflon for the microphone to the stem of the T tube were made The smaller size T tube provided better sensitivity the sound being produced in a smaller volume connected to the condenser microphone (Fig 27 b and c)

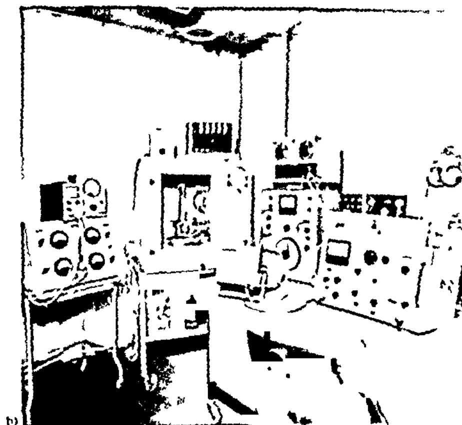
The signal or energy source should be basically the same as in the electrophonic experiments on humans i e it should consist of an oscillator and a DC polarizing arrangement with attenuators ampli fier and correct loading

In addition an analyzing equipment consisting of a transducer (condenser microphone and hydro-phone) with amplifier and a Wave Analyzer was provided A block diagram and a photography of the apparatus are shown in Fig 27

In order to simplify the analyzing of the produced sound the oscillator was modified to be used in con

Fig. 27 Block diagram (a) and photography (b) of apparatus for the investigation of sound production by electric current passed through electrolytes contained in T-tubes (c)





nection with Radiometer Wave Analyzer model SRA producing exactly half the frequency of which the analyzer was tuned to pick up. In this way it was possible to vary the frequency in order to find any resonances, always keeping a narrow bandwidth for the analyzer, thereby increasing the signal to noise ratio substantially. The influence of the first harmonic stray field could in this way be avoided when studying the second harmonic.

Parameters to be studied

Among the many parameters that were studied, the following were considered the most important:

1) Mechanical dimensions responsible for the resonances in the vessel of the liquid and in the volume of air in a coupler, i.e. between the microphone and the surface of the liquid, such as the diameters and length of glass tubes, volume between the surface of the liquid and the microphone
2) Various types of electrolytes (different salts and different solvents and different concentrations)
3) Different types of electrodes
4) Distance between electrodes
5) Temperature
6) The electrical parameters. Voltage (electric field), current (current density) and impedance

Fig 27 c T tubes

A number of pilot experiments seemed to indicate that the current strength was the best electrical parameter to describe the sound production as a function of the input electrical energy

Resonance effect caused by the volume between the surface over the liquid and the microphone was easily demonstrated when the distance between the liquid and the microphone was increased beyond approximately 2 cm As long as this volume was kept sufficiently small it did not give rise to any disturbing resonances in the actual frequency range

Resonance effects are however always present (Fig 28) probably caused by the dimensions of the vessel containing the liquid and of the space between the surface and the microphone

In an electrolyte of constant concentration and temperature the recorded sound seemed to depend mainly upon the current strength and to be independent of the shape and the size of electrodes and the distance between them

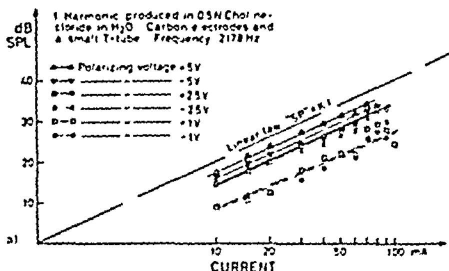
Effect of Polarizing Voltage

Assuming that the transducing mechanism is governed by a square law the introduction of a polarizing voltage superimposed upon the sinusoidal voltage should result in certain sound products which analyzed should prove the existence of the assumed square law (See chapter 5 2.5 on page 34)

By means of the same apparatus as used in the human experiments polarizing voltage (maximum value ± 40 V) was superimposed on the alternating signal supplied to the electrodes of one of the T tubes The experiments were performed using platinum as electrodes and also using carbon electrodes It was easy to show that the second harmonic sound was independent of the applied polarizing voltage It was however much more difficult to make obsevations of the first harmonic sound It was completely impossible to use a hydrophone for these experments because of the electric stray field All measurements were carried out by means of the arrangement with a ½ condenser microphone in the stem of the T tube

Applying DC potential to the electrodes made the system very »dynamic» causing changes at the electrodes and in the liquid introducing gas bubbles

Fig 28 Sound pressure level as a function of applied frequency indicating resonance effects of the T-tube most distinct in the small T tube



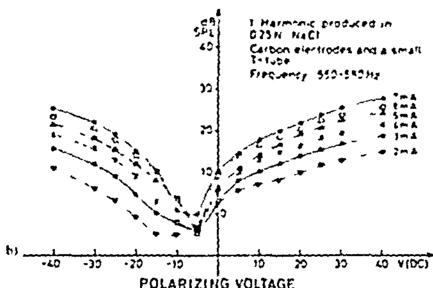


Fig. 29. Sound pressure level as a function of *a) current strength of a 2178 Hz sinusoidal signal for various polarizing voltages* b) polarizing voltage for various current strengths of a sinusoidal signal 550-590 Hz

and polarized electrodes. In Fig. 29 is shown some examples of recorded sound as a function of applied polarizing voltage with the current strength as the parameter (Fig. 29 b) or vice versa (i.e. sound pressure level as a function of current strength and polarizing voltage as parameter (Fig. 29 a)). Within certain limits it certainly was possible to show that the produced first harmonic sound was a linear function of the sinusoidal field and of the polarizing voltage. The influence of the polarizing voltage was slightly less than predicted by the linear law when applying a positive voltage to the system and slightly larger when applying a negative voltage. It also appeared that for certain negative polarizing voltages in the range 0 to minus 15 V at least under special conditions the first harmonic sound dropped below the noise level of the system (Fig. 29 b).

The purely mathematical treatment of the problem of polarizing voltage does not take into account the physical and chemical limitations of the process expressed in the »boundary conditions«. For the present work it was found necessary to look further into these problems which experimentally could be more effectively studied by means of a square wave superimposed voltage upon the sinusoidal source (a square wave is supposed to cause less polarizing effect on the electrodes – less »dynamic« conditions). The experiments on the polarizing effect was considered to have proved the presence of a square law transducing mechanism

Location of the transducing mechanism

One of the most important questions to be answered was Where in the liquid did the transducing mechanism take place?

One possibility was at the boundary between the electrode and the liquid

In earlier works especially the Russian scientists examined this possibility investigating whether the contact area between the electrode and the liquid did influence upon the electrophonic sound (Gersuni and Volokov 1937 and others) They concluded their experiments to give a negative answer to this question

Their experiments were not considered to be convincingly conclusive Therefore the possible location at the electrode (first possibility) had to be reexamined

The second possibility was the remaining surface of the liquid i e the boundary between the liquid and the vessel or between the liquid and the air especially if air bubbles might be contained in this area This possibility might probably be studied together with the first mentioned possibility

The third possibility was the bulk of the solvent

At the surface of the electrodes — or of the liquid

First and second possibility

The experimental arrangement was originally designed in order to answer the first question however it was found to be useful for evaluating the second possibility also

Four T tubes were arranged in such a way that three of them were connected in series and the fourth in parallel as shown in Fig 30

The connection between the T tubes consisted of a narrow PVC tube the inner diameter 1 5 mm length approximately 50 mm The idea was that such a connection constituted an acoustic attenuation («filter») which should attenuate a possible sound produced in the two T tubes containing the electrodes to the middle tube without any electrode If the sound was produced at the electrodes there should be a substantial difference in recorded sound pressure level in the three T tubes connected in series

The effectiveness of the PVC tubes to attenuate sound in the actual frequency range was controlled by means of a bone vibrator pressed to one of the T tubes containing the electrodes and analyzing the elicited sound pressure in the T tubes in the usual way by means of a 1 B & K condenser microphone in each of the stems of the T tubes

The measurements showed an attenuation of at least 25 dB to be secured by the arrangement However since it was not a complete analogy between the vibrations impressed on the liquid from outside and the sound produced inside the vessel a forth T tube was connected to the tube T_C as shown in the figure

The other end of this T tube T_D was closed with a rubber stopper containing an electrode of the same type as used in the two other T tubes however this electrode should in the first experiment be disconnected

The electrical impedance of the three T tubes connected in series was rather high and it was therefore difficult to obtain as high a current strength as in earlier pilot experiments with only one T tube However using an electrolyte with rather good conductively 0 5 M NaCl solution it was possible to obtain sufficient current strength to have a nicely recordable sound production

The experiment showed that no significant difference could be detected in sound level in the three T tubes connected in series (Fig 31) In the

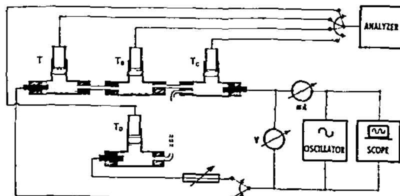


Fig 30 Block diagram of the arrangement of 4 T tubes with microphones T_A T_B T_C in series and T_D in parallel with T_C proving that the sound production takes place in the bulk of the electrolyte when a sinusoidal signal is passed through the tubes

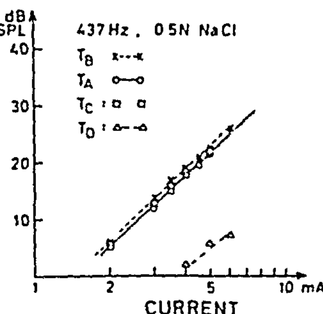


Fig 31 Sound production in the three series coupled T tubes T_A, T_B and T_C as a function of current (mA) and a signal frequency of 437 Hz in a 0 5 NaCl solution. (No significant difference between the sound pressure level in the three T tubes was observed)
In the dead T tube T_D the low sound level picked up during one experiment is considered to be acoustic leakage from T_C. Bending the connecting PVC tube made the sound disappear and in other experiments no sound was picked up from T_D

fourth tube without any current through it no sound could be picked up (but that acoustically transmitted from the neighbour T tube) The result showed that the transducing mechanism could not be located in the boundary surface between the electrodes and the liquid It also was very unlikely that the boundary surface between liquid and air/glass vessel could be the place since a variation in these surface areas in the middle T tube did not cause any other change in sound pressure than to be expected from the variation in air volume between the microphone and the liquid

Most likely the bulk of the electrolyte was the location of the transducer In order to provide further support for this conclusion experiments [illegible]

[illegible]

Location in the bulk of the electrolyte

Third possibility

In these experiments a solution of 0 25 M NaCl was used The current through the tubes was varied from zero up to 6 mA at which value the voltage across the tubes was 150 V

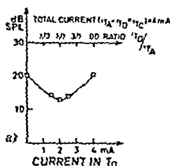
As it appears from Fig 31 the maximum sound pressure level registered by the microphone was approximately 25 dB varying somewhat with the temperature in the solvent The lower the temperature the lower the sound pressure level a variation of 10°C caused a variation of approximately 3 dB The sound pressure level in these experiments with more T tubes connected in series was much higher than in earlier experiments using only one T tube when taking the applied current into account The only explanation for this difference seemed to be the difference in the pathway of the current In the experiments with three tubes connected in series the current was forced through rather thin PVC tubes increasing the current density which means increasing the field strength A closer examination of the influence of the current density upon the sound production was carried out by means of the parallel connected T tube the electrode of which was connected to the oscillator through a potensiometer (see Fig 30)

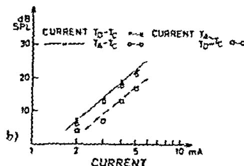
In this way it was possible to vary the current density through the two PVC tubes entering one end of the T tube T_c, without varying the total current through this T tube as measured in the wire connecting the electrode in the other end of the vessel to the oscillator The sound picked up in this T tube T_c as a function of the ratio between the current in the two parallel connected PVC tubes is shown in Fig 32 It indicates that when the total current through the T tube was kept constant the sound production reached a minimum when the current was the same in the two parallel connected PVC tubes This confirms the earlier suggestion that the sound production was a function of the current density – the field strength – since the minimum field strength was obtained when the current was the same in the two PVC tubes while any unbalance in current through these tubes increased the field strength in one of them

A significant increase in the sound production was observed when the electrode in the T tube T_c was disconnected from the oscillator and the current was passed through the T tube T_D and only through the

Fig 32 a) Sound production in T-tube T_C as a function of *the current (iT_D) in one of the* parallelcoupled T-tubes (T_D) *(or of the ratio between* iT_D and iT_A) when the total current in T_C is kept constant (4 mA) Minimum sound production when the current is the same in the two parallel coupled T tubes
b) Sound production in T-tube T_C as a function of *current (mA) when the current* passes through the PVC tube *from* T_A (o – o) or from T_D (x – x) or equally from both of these two T-tubes (□-□)

SOUND PRESSURE MEASSURED IN T_C
435-440 Hz 0 25 N NaCl





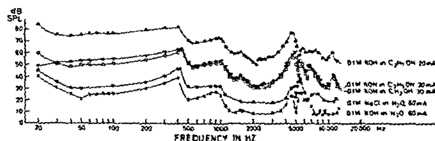
one end of the T tube T_C to the T-tubes T_B and T_A (see Fig 30) This indicates that the main sound production in T-tube T_C *took place in the area* where the two PVC-tubes entered the liquid in one end of the T-tube and where the current density, i e , *the field strength was increased compared with* the earlier pathway of the current

The results of these experiments permit the *following conclusion The sound production* took place in the bulk of the solution, and excluded the boundary area between the electrodes and the solvent, and also the surface of the liquid (boundary to air and glass vessel) It also showed that the current density was of importance for the transducing mechanism

Various types of electrolytes.
The most sensitive system with a small T tube and the large size carbon electrodes were used to analyse the sound production in different types of electrolytes The produced sound as a function of *frequency at constant current* was recorded by means of a Brüel & Kjær Level Recorder, Type 2305 connected to Radiometer Wave Analyser, Type FA3, and the *results for different* types of electrolytes are shown in Fig 33 A couple of resonances are most likely caused by the acoustic conditions in the vessel or by the structure of the spesific electrolyte Although it may be of interest to investigate closer these effects, it was considered unnecessary to do so in the present study

The conclusion of these experiments was that the type of electrolyte, i e the kind of salt and the type *of solvent used, together with the molar strength of* the solution are of importance for the transducing mechanism

Fig 33 Sound production in a small T tube as a function of frequency for different types of electrolytes For each electrolyte the current was kept constant In the case of isopropylalcohol (C_3H_7OH) a current of 2 mA was used whereas the curve has been drawn for 20 mA in order to separate it from the other curves



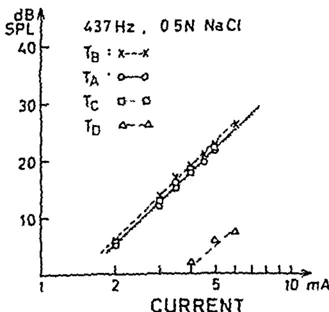


Fig 31 Sound production in the three ... T tubes ...

... sound pressure level in ... tubes was observed)

In the dead T tube T_D the low sound level picked up during one experiment is considered to be acoustic leakage from T_C Bending the connecting PVC tube made the sound disappear and in other experiments no sound was picked up from T_D

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Most likely the bulk of the electrolyte was the location of the transducer In order to provide further support for this conclusion experiments with the same arrangement of the four T tubes were ...

Location in the bulk of the electrolyte.

Third possibility

In these experiments a solution of 0.25 M NaCl was used The current through the tubes was varied from zero up to 6 mA, at which value the voltage across the tubes was 150 V

As it appears from Fig 31 the maximum sound pressure level registered by the microphone was approximately 25 dB, varying somewhat with the temperature in the solvent The lower the tempera ture, the lower the sound pressure level, a variation of 10°C caused a variation of approximately 3 dB The sound pressure level in these experiments with more T tubes connected in series was much higher than in earlier experiments using only one T tube, when taking the applied current into account The only explanation for this difference seemed to be the difference in the pathway of the current In the experiments with three tubes connected in series the current was forced through rather thin PVC-tubes, increasing the current density, which means incre asing the field strength A closer examination of the influence of the current density upon the sound production was carried out by means of the parallel connected T tube the electrode of which was connected to the oscillator through a potensiometer (see Fig 30)

In this way it was possible to vary the current density through the two PVC tubes entering one end of the T tube T_c without varying the total current through this T tube, as measured in the wire connecting the electrode in the other end of the vessel to the oscillator The sound picked up in this T tube T_c as a function of the ratio between the current in the two parallel connected PVC tubes is shown in Fig 32 It indicates that when the total current through the T tube was kept constant, the sound production reached a minimum when the current was the same in the two parallel connected PVC tubes This confirms the earlier suggestion that the sound production was a function of the current density – the field strength – since the minimum field strength was obtained when the current was the same in the two PVC tubes while any unbalance in current through these tubes increased the field strength in one of them

A significant increase in the sound production was observed when the electrode in the T tube T_c was disconnected from the oscillator and the current was passed through the T tube T_D and only through the

of the beam of the used Oscilloscope This was done by manually increasing and decreasing the pressure in a small volume connecting the microphone to a Politzer bag

A Tektronix Storage Oscilloscope, model 564 was used for the phase experiments applying the microphone voltage to the upper beam and the electric field to the lower beam In figure 35 is shown the results at a frequency of 270 Hz The same picture was found at other frequencies, when corrections were made for the phase shift caused by the apparatus It was evident from these measure ments that when an alternating current was passed through the electrolyte, the volume of the liquid was increased each time the electric field had its maximum value either negative of positive These volume variations were the origin of pressure variations, i e the sound field in the liquid and above its surface, characterized by having twice the frequency of the applied alternating current In addition the phase relation between the sound field and the applied electric field was measured by means of an Oscilloscope with only one beam on which the electric field was fed to the horizontal plates and the microphone potential to the vertical plates, and the produced Lissajous figure examined The resulting Lissajous figure had a shape of the upper part oof a circle or ellipse, in agreement with the earlier observations (⌒)

It therefore seems correct to conclude that experimental results are in agreement with the hypothesis

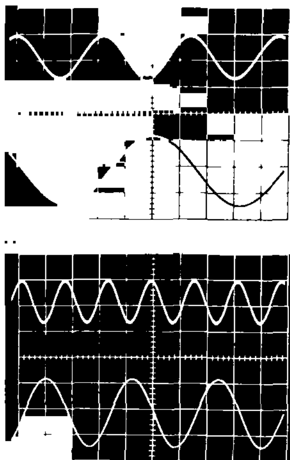


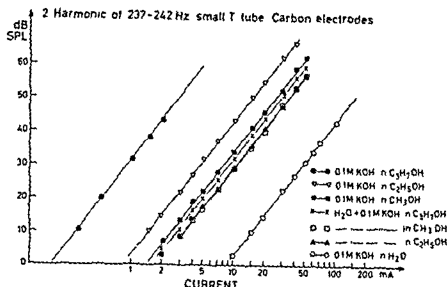
Fig 35 The phase relation between the recorded «electrostriction» sound (upper beam) and the applied electrical field (lower beam) at a frequency of 270 Hz using two different sweep times

Discussion And Further Supporting Experiments
In order to add further experimental evidence to this apprehension it shall be reported some considera tions resulting in new experiments

If the electrodes were given a fairly large plane surface facing each other in a parallel position the electrodes should be caused to vibrate according to the condenser effect described earlier in the large area electrode chapter 5 2 under paragraph 5 2 7 1 These vibrations should cause the liquid to be compressed each time the field had its maximum positive or negative value i e counteract the simultaneous increase in pressure caused by the loosening of packing around the ions solvation sheaths i e the two sound producing effects should be 180° out of phase

(Earlier exppenments reported on page 49 «At the surface of the electrodes—» have demonstrated that vibrations of electrodes are not responsible for the studied sound production The very special case now taken into account of course does not contradict the earlier proved statement)

In a complete homogenous electric field the acting force between the electrodes is independent of distance between electrodes as long as the current is kept constant However, with shorter distance between the electrodes the caused pres sure per volume unit will be greater In addition the field will not be completely homogenous thereby increasing the force at shorter distance between the electrodes Both these effects should decrease the



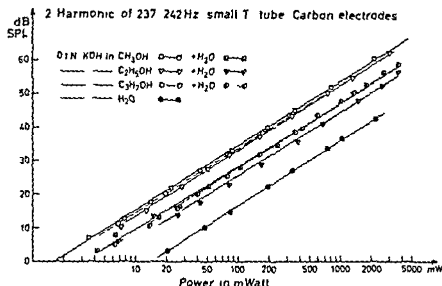


Fig 37 Sound production (SPL dB) in a small T tube with carbon electrodes when passing a sinusoidal current (237 242Hz) through different solutions of 0 1N KOH
a) as a function of current
b) as a function of power
Water is seen to be a poorer transducer compared to the alcohols as solvents When water is added to the alcohols their transducer efficacy deteriorate

DC source or produced by the system itself (different contact potentials between electrodes and liquid and possible rectifying effects etc) adds a first harmonic sound according to a square law mechanism governing the transducer Mathematically it is described by the equation Sound pressure $p = k_1 I^2 = k_v V^2$

It is of course of interest to study the transducer constant k in more detail However it was not considered appropriate to do this in the present work Some indication about the variations observed in this constant shall however be reported

When observing the produced sound as a function of current strength the consentration (molar strength) is governing the transducing mechanism in delute solutions up to a certain consentration A further increase of the molar strength does not influence upon the transducing mechanism This is shown in Fig 38 for NaCl solution which is representative for the general effect observed in other types of solutions

The variation with molar strength may be considered as an indication that the conductivity of the electrolyte is of vital importance to the effect lower conductivity (higher impedance) making it possible to obtain a higher field strength in the liquid thereby increasing the effect upon the ionic field which in turn should influence the size of the volume changes resulting in higher sound pressure levels However another possibility is that the fewer the ions the more effect of the outer electric field on the ionic field thereby increasing the volume variations of

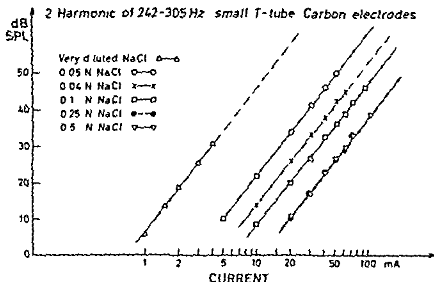


Fig 38 Sound production (SPLdB) in a small T tube with carbon electrodes as a function of current for different consentrations of NaCl in water Increasing the molar strength beyond N–0 25 does not influence upon the sound production as a function of current

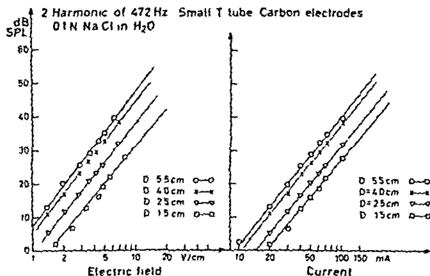


Fig 39 Sound production (SPLdB) in a small T tube with carbon electrodes as a function of
a) electric field (V/cm)
b) current (mA) for different distances (D/cm) between electrodes Longer distance results in higher sound production for the same field strength (V/cm) probably because more solvation sheaths are involved in the volume (pressure) variation

ionic shells In order to show the effect of variation in the electric field the sound production as a function of the field is presented in Fig 39

A third possibility has also to be considered i e the degree of ionisation

Only in very dilute solutions the activity coefficient approaches 1 and the ionization is complete The suggested mechanism for the sound production volume variations of primary solvation sheats presupposes a sufficient number of dissociated ions in the solution

This condition is probably fullfilled in the actual solutions in the present work It may also be possible that the transducing mechanism (to a certain degree) is working although a complete dissociation has not taken place A «saturation» of the sound production as a function of current does take place (at a molar strength of 0 25 for NaCl in water) however the transducer mechanism was not observed to vanish a higher molar strengths A distinct increase in sound production as a function of current was observed in very diluted solution – see Fig 38

Most probably the discussed three possibilities influencing upon the transducer constant represent mechanisms interacting with each other The existence of volume variations of primary solvation sheaths is however considered to be demonstrated in the reported experiments indicating that such a mechanism is the main origin of the sound production

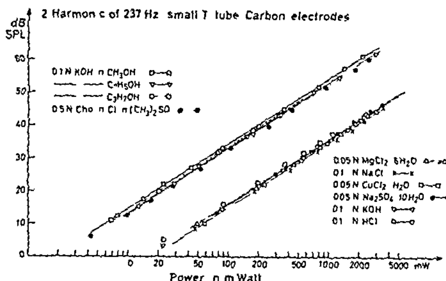


Fig. 40. Sound pressure level (SPL dB) in a small T tube with carbon electrodes as a function of electric power for various types of liquids. A distinct difference is observed between alcoholic (organic)- and aqua solutions, the latter being the poorer transducers.

Of special interest is the study of energy transformation in the transducing mechanism already partly discussed in chapter 5.3.7.1.

In Fig. 40 is shown the sound pressure level as a function of input electric power to the liquid for different types of liquids and for different consentrations. It appears to be a very distinct difference between two groups of solvents: the aqua solutions which are the less efficient transducers and the none aqua solutions (organic liquids as alcohols and dimethylsulfoxide) being much more efficient.

Only small differences were observed within each of the two groups. These differences may be caused by not having had the temperature completely constant all the time and may also be due to the fact that minor variations between different solutions really excist. In summing up it may be concluded that the sound pressure level obtained in the liquid is a function of the type of solvent used, of the molar strength in dilute solutions up to a certain limit, and of the current density (or the field strength) of the alternating current and of the presence of a polarizing voltage. It also is a function of the temperature and may be influenced by possible air bubbles in the system.

Conclusion with respect to the electrophonic effect

An effect depending upon so many variables – which in the electrophonic experiments on humans have been little acknowledged and poorly controlled – is to be expected to cause reports of rather varying descriptions with respect to intensity and quality of the perceived sound.

The results of the model experiments in T tubes seem to be able to explain all the different results reported from human experiments. They exclude the earlier proposed transducing mechanisms located to the middle ear (eardrum – promontorium condenser system) and/or inner ear structures (piezo electric effects, vibrating membranes etc.).

The transducing mechanism in the electrophonic effect in the Brenner method is located in the bulk of the electrolyte filled into the ear canal in which the electrode is immersed. The produced variation in pressure in the liquid sets the ear drum into vibration and may also effect the bone surrounding the ear canal transmitting the vibrations to the inner ear by means of bone conduction. The perception of first and/or second harmonic sound in this system is elicited in the regular way for vibrations entering the inner ear either through the stapes foot plate or through vibrating bone structures or a combination of both routes.

5.4 Middle-ear electrode

Fromm, Nylén and Zottermann (1935) were probably the first to use an active electrode on the mucous tissue inside the middle ear when they tried to pick up aural microphonics. During their experiments they also in «suitable cases» elicited electrophonic perception by applying an alternating current to the same electrode system. However a systematic study was first carried out in 1940 when Jones, Stevens and Lurie (1940) used this type of electrode to examine 20 ears lacking tympanic membrane. They thought Brenners method unsafe for such ears. As said Stevens (1937) «We just

couldn t fill those ears with a saline solution ears full of scar tissue or suppurating from cronic otitis media» Their basic idea for studying the electrophonic effect in such ears was the theory proposed by Stevens and Jones (1939) that the eardrum promontorium formed a condenser transducing mechanism responsible for the electrophonic effect in the Brenner method governed by a square law In ears without eardrum the square law response was not expected to be found Jones et al (1940) confirmed this however, they did not use the same method (Brenner's) in ears with normal or pathological middle ear and therefore their conclusion may not necessarily be correct

The object of the present work was to examine their experimental observations and reconsider their theory taking into account the possibility of «new» effects caused by the «new» electrode system applied to the pathological ears

5 4 1 Apparatus and Procedure

The apparatus was similar to that used in the Brenner method and was copied from the work of Jones et al (1940)

A block diagram of the apparatus is shown in Fig 2

The indifferent electrode was as usual clamped to the wrist and the active electrode consisting of a copper or brass rod with a tuft of cotton dipped in physiological salt solution was inserted through the ear canal and placed somewhere on the promontorium A headband with a holder for the electrode kept it positioned against the wall of the inner ear

Subjects were chosen among patients in the ENT department of Rikshospitalet lacking eardrum but having a well preserved bone conduction threshold

The patients were instructed to compare the sound they might hear when an alternating current was passed through the system with a pure tone to the other ear or make observation of beats – according to the method described in chapter 4

It was however found very difficult to perform these experiments because it appeared to be rather boring for the patient

The main goal for the present experiments was therefore to confirm or disprove the results reported in the work of Jones et al (1940)

5 4 2 Auditory Response

The threshold of discomfort was found to be rather low «Unwanted» responses from other nerves in this region of the middle ear might be elicited such as the vestibular nerve (the subject might become dizzy) or efferent facial nerve fibres to muscles (eliciting muscle twitches in the face) or the subject reported feeling of a burning or electrical shock

It appeared to be rather difficult to elicit a hearing sensation of a pure tone Some kind of noise feeling of vibrations sensation of a pure tone often together with a noise but many times no sensation at all

The description of experimental results of Jones et al (1940) was found to be in complete agreement with the experimental results of the present work on three patients

Because the experiments were boring for the patients and since the results did not seem to bring about any new aspects of this type of electrophonic hearing it was found unnecessary to trouble more patients obtaining results from a larger material The report of Jones et al (1940) of experiments on 20 pathological ears is considered representative for this kind of study

Of 20 ears examined 9 heard pure tones corresponding in pitch to the frequency of the applied voltage 5 heard «buzzing» noise the character of which was roughly independent of the stimulating frequency 2 heard both the pure tone and the buzzing noise although at different frequencies and 4 heard nothing

Considering the possibility of a direct stimulation of the structures in the inner ear in these ears lacking eardrum it was judged to be important to examine what kind of sound perception resulted of the Brenner method on such ears

Such experiments were carried out in one young patient with a radically operated ear He was trained as an acoustical observer Before the ear was filled with a NaCl solution it was sprayed with antibiotics A silver silverchloride electrode was inserted in the NaCl solution His auditory response was exactly the same as in normal ears using the Brenner method

5 4 3 Threshold

The findings of Jones et al (1940) were confirmed with respect to the reported difficulties in deter

mining reproducible electrical threshold curves Only as long as the electrode was not moved there was a possibility of reproducing the threshold values In the present work it was found very difficult to obtain threshold values for a sufficient number of frequencies to produce a complete thres hold curve Jones et al (1940) reported that only five of their subjects were able to give consistently reproducible threshold curves and blamed the others for not being sufficiently good observers With the experience from the present work it is suggested that the unstability and transient charac ter of the sound effect is an equally important explanation

For this reason together with the earlier mentioned attitude not to bore patients with unjustified measurements no threshold curve from the present work is presented for the middle ear electrode system

It was considered more valuable to produce an electrical threshold curve for the Brenner method in an ear lacking eardrum than obtaining a fortuitous threshold curve for a particular placement of the middle ear electrode in the same ear

In using the Brenner method the subject also experienced boring effects when the electrical signal was below 750 Hz It was therefore not attempted to determine threshold values below this frequency

The electrical threshold curve for the Brenner method in a radically operated ear is presented together with the best threshold curve obtained by Jones et al (1940) with the middle ear electrode (Fig 41)

5 4 4 Loudness of the Produced Sound

It was difficult to obtain a greater loudness than corresponding to about 20 dB sensation level compared with an air conducted pure tone to the other ear

The experiment was too uncomfortable for the patient to justify thoroughly examining of the loudness function

5 4 5 Effect of Polarizing Voltage

The effect of polarizing voltage was only studied so far that the reported results of Jones et al (1940) were qualitatively veryfied The pitch of the elec trophonic tone was not markedly alterated by a small positive or negative voltage superimposed upon the AC signal

The conclusion of Jones et al (1940) that the transducer acts according to a linear law seems correct

5 4 6 Location of the Transducer

If as Jones et al (1940) proposed the tone perception is the result of a proper electrical stimulation of certain structures within the cochlea (the polarized Reissners membrane as proposed by Hallpike and Rawdon Smith (1934) or the haircells of the organ of Corti as proposed by Jones et al

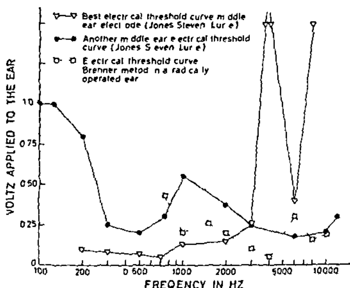


Fig 41 Electrical threshold curves in two patients using the middle ear electrode one curve representing the smal lest voltages used compared with other cases (Jones et al 1940) and one patient with a radically operated ear using the Brenner method The similarity between the curves representing the two different electrode systems is seen to be quite good

(1940)) then the response pattern of those elements to electrical stimulation of various frequencies should depend on two main factors – before the neural mechanism is taken into account

1 The mechanical response of each element (polarized membrane or piezo-electric structure) to a given voltage
2 The voltage distribution along these elements as it is established for a particular placement of the electrode

The first factor reflects the inherent characteristics of a specific cochlea and cannot be influenced by different electrode systems or variations in the placement of a particular electrode

The fact that a patient with a radically operated right ear did not experience the same type of tonal sensation when the electrical signal was supplied to this ear by means of the Brenner method as by a silver silver chloride rod and a small tuft of wet cotton placed upon the scar tissue in the middle ear cavity seems to exclude stimulation of such elements as mentioned as factor 1

The second factor depends upon the placement of the electrode and upon the electroanatomy of the cochlea According to Bekesy (1951) the electrical model of the cochlea is a transmission line – consisting of the two scalas separated by the cochlear duct In obtaining the electrophonic threshold curve the voltage at the round and oval windows respectively determines the result However it is the voltage between the electrodes that is measured and used as threshold value in the electrophonic experiments

A change in the position of the active electrode might cause a change in voltage distribution at the two windows and a new threshold curve might therefore result However it is possible to show mathematically that if two such curves have two or more points in common both curves should be identical The experimental results are not consistent with this If the active electrode is moved slightly to a new position it may be impossible to elicit a hearing sensation or the new threshold curve may be completely different from the old one crossing it at several points as shown in the work of Jones et al (1940)

These experimental facts indicate that the transducing mechanism cannot be located inside the cochlea

If we assume that the transducing mechanism is outside the cochlea f i at the contact point between electrode and the tissue then there are two important factors that are changed by moving the electrode to a new position

1 The ability of the tissue to act as a transducer
2 The acoustical and/or mechanical impedance from the sound source to the inner ear

When the placement of the elctrode is changed one may expect a change in the transducing mechanism and in the transmission line for sound and vibrations to the inner ear Therefore all kinds of irregularities in threshold curves may be caused by moving the electrode from one place to another

The fact that application of the electric energy through liquid making contact with the entire area around the promontorium causes a definite change in the quality of the perceived sound from first harmonic to dominating second harmonic strongly points away from the inner ear as the locus for the transducer

The concept of a periferal location led to model experiments Leather soaked with water and a small size electrode either with or without a tuft of cotton at the tip was placed upon the leather surface The indifferent electrode was placed in the water into which the lower part of the leather was descended In this system an alternating current was producing a sound of the same frequency as the alternating current

It is assumed that the same kind of transducing mechanism was responsible for the perceived sound in the middle ear electrode system

The sound production was easily located to the contact between the electrode and the wet leather (tuft of cotton) It is a faint sound production and it is not possible to increase it very much because the properties around the electrode are rapidly changing when the energy is increased In the model experiments the produced sound was radiated as air conducted sound and also transported as structure born sound

It is assumed that the same two routes are used in the propagation of sound from the electrode placement in the middle ear electrode system i e air conducted sound is picked up by the oval or round window membrane and bone conducted sound is picked up by the promontorium wall

5 4 7 The Transducing Mechanism

Linear electrocapillary transducers are described in the physical litterature f i by Freundlich (1930)

The physical principle for the sound production is a variation in surface tension according to the polarity

5 4 7 1 Experiments with electrocapillary transducers

In order to verify this some experiments were carried out in line with those described by R Sabine (1876) A container with mercury served as the «in different» electrode for a drop of water at the surface of the mercury A thin platinum wire inserted in the drop acted as the «active» electrode The signal source was either rel iy operated DC supply producing rectangular waves or a low frequency oscillator A microscope was used together with stroboscopic illumination (General Radio) in order to study the possible pulsation of the drop A schematic drawing of the apparatus is shown in Fig 42

When the drop was made positive its hight increased and it was shaped like half of a ball as a result of increased surface tension

With the opposite polarity (the drop made negative) it deflated because of decreased surface tension

By means of the stroboscopic illumination it was possible to control the frequency of the pulsating drop when an electrical signal in the audio fre quency range was applied The resulting sound could also be picked up by a stethoscope The observed pulsations and the pitch of the perceived sound corresponded to the frequency of the applied alternating voltage So the transducing mechanism obeyed a linear law

The transducing mechanism was little efficient and it was easily destroyed For instance when attempting to increase the sound production by in creasing the electric energy to the system the water started to evaporate and the pulsations ceased Other unknown causes m iy be related to the duration of the experiment or to some chemical processes etc , could destroy the transducing mechanism

Changing the mercuury with br iss or leather made the effect much less pronounced either this was caused by a decrease in the transducing mechanism or in the possibility to observe it On leather it was impossible to provide a single drop of water, because it imidi itely spread out Using a thin electrode pressed into the very wet leather it sometimes was possible to produce a first harmonic sound This transducing mechanism may possibly be present in the Brenner system resulting in a first harmonic sound production on certain occasions (some times a first harmonic sound could be observed without being cancelled by any polarizing DC superimposed on the electrode)

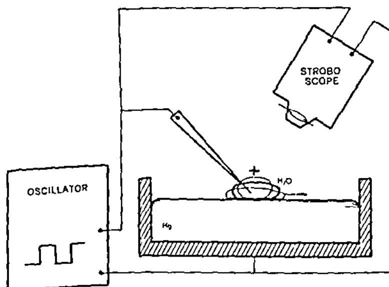


Fig 42 Schematic d awing of apparatu used to study the sound product on in a drop of water due to variat on in the surface tension with the po larity When the drop is made positive the surface tension is increased and the drop is shaped like a ball whereas negative polarity ca ises a decrease in surface tension and the drop flattens Such pulsations eliceted by a sinusoidal voltage to a l quid may cause the radiation of a tone corresponding in pith to the frequency of the sinuso d

5 4 7 2 *Conclusion with respect to the electrophonic effect*

In the middle ear electrode system the condition for the surface tension transducing mechanism to cause a hearing sensation is that the varying capillary forces act upon suitable structures causing vibrations to be transmitted to the inner ear Such structures may in some cases be the wet piece of cotton wool rapped around the electrode In other cases scar tissue in the promontorium area in contact with the electrode may contain liquid the surface tension of which may be caused to vary producing vibrations to be transmitted to the inner ear as bone conducted sound and also as air conducted sound

The vulnerability of the effect together with its special requirements to the surroundings are probably the reason for the instability of the threshold found in the middle ear electrode system and also for the peculiarly shaped threshold curves recorded

The final conclusion is therefore that the mucous tissue on the promontory wall and the type of scar tissue found in a middle ear with sequela otitis may act as a transducer transforming the variations in capillary forces (surface tension variations) into vibrations according to a linear law

If this is correct then probably the lining of the inner part of the meatus wall also might have this ability A check of this hypothesis led to the development of the meatus electrode

5 5 Meatus Electrode

In addition to the purpose of checking the hypothesis about the mucous tissue to act as a transducer and transmitter for audible vibrations the idea behind the meatus electrode was to use a system in which the probability was very small that the electrical signal should pass through the inner structures of the cochlea First being aware of the effects of different electrodes in electrophonic hearing it seemed very natural to try to prove that the perceived sound was produced peripheral to the cochlea as accounted for in the author s paper of 1953 I was considered that if at all it would be possible to produce a first harmonic sound with a meatus electrode then an explanation of the transducing mechanism in the middle ear electrode system by means of structures *in the inner ear should be quite unnecessary*

5 5 1 *Apparatus and Procedure*

The indifferent electrode was usually strapped to the forearm However in one series of experiments the indifferent electrode was formed of a coaxial shielding surrounding the active electrode so that it touched the meatus lining along a circle 1 mm outside the active electrode

The purpose of this was to try to prevent the electric field from spreading too far out i e from reaching the cochlea This electrode system can hardly be considered to satisfy the definition of electrophonic hearing since the electric current does not pass through the head

The active electrode was usually a metal rod or a ball whose diameter was less than 1 mm In some experiments a thinner electrode was used the tip of which was wrapped with a piece of absorbent cotton soaked in a salt solution

The meatus was cleaned of ear wax and the active electrode was pressed against the wall about half way between the opening and the eardrum The subject held the electrode in some of the experiments in others it was fixed and the pressure was varied by means of a screwing device of the type used to measure inside diameters

It was necessary to vary the place of contact between the electrode and the meatus wall in order to find a suitable place for sound production

5 5 2 *Auditory Response*

When a sinusoidal signal was applied to this system it was usually possible by moving the electrode around to find a point at which the subject could hear a tone There were individual differences among the eight persons investigated in the ease with which a hearing sensation could be elicited and also in the sensitivity of the various areas on the meatus lining of each listener The electrode with the wet cotton tuft appeared to be most effective particularly in preventing effects of the electrostatic type from occurring (large area electrode system)

Two different responses occurred Listeners whose hearing sansation was easily elicited heard a pure tone whose *pitch corresponded to the* frequency of the alternating current This was particularly likely *with the wet cotton electrode* Listeners whose response was hard to elicit heard a complex tone containing first and second harmo-

nics This was especially true when a metal electrode was applied loosely to the meatus lining However this second harmonic response may be considered to be an unavoidable side effect because the electrode although presenting a small contact surface may under certain conditions exhibit the electrostatic effect of the large area electrode

5 5 3 *Threshold*

In parallel to the experience with the middle ear electrode only certain placements of the electrode resulted in an auditory response to the stimulating current and the areas tested were not efficient for all frequencies The threshold curve was therefore zigzag It is very difficult to obtain a detailed curve with the electrode in one position because of the discomfort of having the electrode in the meatus for a long enough time In addition when it was necessary to increase the voltage in order to produce a hearing sensation the stimulating current evoked a number of undesirable side effects such as pain twisting of muscles etc The resemblance to the threshold curves for the middle ear electrode was very evident

For frequencies below 150 Hz the senstion was not tonal but rather one of noise or vibrations inside the head In some instances both the vibration and the pure tone could be experienced at the same time (for frequencies in the transition range)

5 5 4 *Loudness of the Produced Sound*

The loudness of the tone perceived was usually very low It never corresponded to more than 25 – 30 dB S L usually only around 10 dB S L for the voltage applied (maximum 15 volts) The threshold of discomfort was rather close to the electrophonic threshold and for some frequencies it was even lower than the hearing threshold It was therefore not possible to obtain any reliable correlation between loudness and applied voltage

5 5 5 *Effect of Polarizing Voltage*

The characteristic feature of the auditory response was the lack of influence of a polarizing voltage upon the tone perceived The observation by means of beats and comparison showed no influence upon the intensity of the first harmonic by the use of any special polarizing voltage Even when the perception was a mixture of first and second harmonics it was impossible to decrease the intensity of the first harmonic by the use of polarizing voltage This demonatrates that the occurrence of a first harmonic component is due not to the polarizing effect of some DC potential upon a square law transducing system but rather to the presence of a linear transducer

5 5 6 *Location of the Transducer*

The sensitivity of the effect to changes in the placement of the electrode indicates that certain structures of the elctrode or of the skin under the electrode are responsible for the sound production and the sound transmission This conception is strengthened by the dependency of the effect upon the current density (only a small size electrode will produce this effect) and also by the fact that changing from a pure metal to a piece of soaked cotton makes it easier to elicit the first harmonic response

The same arguments as claimed for the middle ear electrode s dependency on placement with respect to the eliciting of the sound perception are applicable in the meatus electrode system The exclusion of the cochlea as the location of the transducer is further supported in this electrode system by the fact that a tone perception could be evoked even with the indifferent electrode surrounding the active electrode preventing the electric field from reaching the cochlea

The similarity between the elicited sound in the two electrode systems strongly indicates the transducing mechanism to be located at the place of the electrode and the vibrations transmitted from this place to the inner ear by means of bone and cartilage and air-conduction

5 5 7 *The Transducing Mechanism*

The demonstration of similarity between the sound production in the meatus electrode system with the middle ear electrode system should be sufficient evidence for the transducing mechanism to be the same in the two electrode systems which basically are identical but for the electrode placement The main transducing mechanism is considered to be the electrocapillary effect as accounted for in the preceding chapter 5 4 7 concerning the transducing mechanism in the middle-ear electrode system

5 5 7 1 *Discussion and Conclusion*

Presupposing a peripheral located transducing mechanism of the electrocapillary type all experimental findings in the last two electrode systems may have their natural explanation The transducing mechanism itself is dependent upon the presence of a liquid the surface tension of which is varied by the variation in the polarity of the sinusoidal signal The liquid must have a location and a surrounding effecting a vibration of structures in response to the variation in the surface tension of the contained liquid It is very likely that this will vary from place to place and also that changes may occur during the course of the experiment making it necessary to find a new placement of the electrode in order to obtain continous sound production

From the place of the electrode the vibrations are transmitted to the inner ear The effectivity of the possible routes for this transportation of acoustic energy may vary from place to place and also vary with frequency in one place This may be the reason for the zigzag form of the threshold curves and the lack of reproducibility of threshold curves in two different placements of the electrode The model experiments on wet leather with auscultation of the produced sound strongly support the proposed explanation

It therefore seems quite unnecessary to assume the existence of a transducing mechanism in the cochlea although the experimental results do not completely rule out the possibility of such a mechanism – however being «masked» by the peripheral located electrocapillary transducer The probability for this is considered to be very small

6. Final discussion and conclusion

According to experiments in the present work and to numerous reports through many decennia and from many countries the following conclusion is evident

When an *alternating current* is passed through the head various kinds of hearing sensations may occur These are dependent upon the types of electrode system applied and upon the properties of the ears tested

I A noise may be heard by subjects with (1) normal ears for frequencies below 150 Hz and (2) operated ears for all frequencies but especially in the range below 50 Hz
The noise heard by normal ears is akin to a feeling of vibration and is most probably due to some kind of muscle stimulation
Operated ears are also subject to vibration but in addition they may also hear noise This noise is more like a hissing sound with resemblance to white noise and is considered to be caused by direct stimulation of the hearing nerve

II Both normal and operated ears may perceive a tone The characteristic features of the tone such as harmonic content intensity transient effect are a function of the electric system in use
It is concluded therefore that the transducing mechanisms involved in the tonal response are several and that their locations are peripheral to the cochlea

This is strengthened by the electroanatomical properties of the cochlea and by our recent knowledge of the cochlear microphonics together with the experimental facts reported earlier f 1 about the variation in threshold curves by variation in placement of the electrode in the middle ear electrode system With the indifferent electrode placed far away from the ear the inner structures in the

the cochlea must be difficult to reach with a proper electrical current even with an electrode placed on the promontorium That some part of the electric energy may enter into the vestibulum of the cochlea and be conducted through nerve fibres and blood vessels in the meatus acusticus internus is possible The response to this route of the applied electric current is very likely the reported noise when the hearing nerve or part of it has been sufficiently excited (This is in agreement with report from surgery on the ear and more or less unintentional stimulation with electricity in this region causing a sensation of noise (Nylen reported by Gisselsson 1950)

The unveiling of transducing mechanisms in electrophonic hearing in the present work makes the peripheral location most probable In this respect there should hardly be any doubt that the fricative sound caused by the moving electrode and the second harmonic sound produced by the large area electrode are produced at the place of the electrode and that the acoustic energy is transmitted to the inner ear and perceived as a sound in the normal way

The middle ear electrode with its sound production similar to that for the meatus electrode together with the explanation of a physical transducing mechanism common for these two systems should be quite strong evidence that even here the sound perception is elicited by vibrations transmitted from the place of the electrode to the inner ear

The same argument should hold for the Brenner method for which the physical transducing mechanism together with the observation of the same type of sound perception in normal ears as in ears lacking an eardrum should make the search of other explanations unnecessary

The newer trend in electrophonic research and theory using an assumed extra sensory neural pathway as a working hypothesis (Puharich Lawrence and Dugot 1969) has not been dealt with in the reported present work

Their main apparatus has however been copied and their method used in some experiments (Puharik et al 1963) It was found that the electrode system was similar to the large electrode and the method very much the same as used by the early «Radio enthusiasts» (1925)

There should be no need for any extra system to explain the effect The «transdermal hearing» (TD hearing) is caused by vibrations transmitted to the inner ear from the place of electrodes The rectifying mechanism of the body demodulating the amplitude modulated signals is considered to be outside the scope of the present work

In a recently published work about evoked auditory sensations by microwave pulses (Guy and Chou 1975) it is suggested that the acting mechanism is a «conversion of electromagnetic energy to acoustic energy due to the thermal expansion in the tissues within the head» A direct electric stimulation of any part of the auditory neural system seems to be excluded A possibility may therefore exist that the TD hearing in addition to vibrations from the electrode also has a component of «thermal expansion»

The thermal expansion theory contains however some points rather difficult to understand and accept For instance the assertion that a raise of the temperature in the tissue of only 5 10⁶ °C should be sufficient to mediate a hearing sensation and the observation that only the high frequency portion of the auditory system seems to be involved in this hearing sensation

The «electrostriction» theory suggested in chapter 5 3 7 2 explaining vibrations in electrolytes under influence of a varying electric field may possibly be applied to the electromagnetic hearing phenomenon This is however outside the scope of the present work as already mentioned in chapter 5 under the «sixth possibility» page 15

The important common point in both the thermal and the electrostriction hypothesis is that a transducing mechanism converts the electrical energy into mechanical vibrations which in turn are transmitted to the inner ear causing a hearing sensation in the «normal» manner for the ear

The use of TD therapy (Puharic et al 1969) in treatment of deaf and hard of hearing patients including cases of Meniere s disease is not considered to need further comments than reminding about the similarity with the electrophonic therapy supported by tsar Alexander I (Brenner 1968) Carefully controlled experiments by unbiassed researchers have shown no difference with respect to hearing between patients treated with TD therapy and those «placebo» treated (Gerken et al 1974 Glattke and Simmons 1974)

Since electrophonic hearing – according to the findings reported in the present work – is constituted by «normal hearing» if a tone is perceived when stimulating with a sinusoidal electric current it

seems at the moment very small probability to use the effect in clinical work or for hearing therapy It could probably be managed to use the electrostriction phenomenon (Brenner s method) to set up vibrations in the perilymph of the inner ear by means of implanted electrodes in the cochlea one at the entrance the other at the top of the cochlea One should expect pressure variations to be elicited in the perilymph and/or endilymph according to the description in 5 3 7 A travelling wave in the cochlear partition might be set up and local pressure stimulation take place and a hearing sensation be elicited in the »normal» way It is however not possible to see that much could be gained by this because it is possible to transmit vibration energy to the inner ear by means of adequate amplification of air or bone conducted signals

The only means of using electrical stimulation in hearing therapy seems to be to stimulate the hearing nerve This has been tried by Blair Simmons (1966) House (1961 1973) and others with rather small success It is easy to stimulate the hearing nerve with electricity and elicit some kind of noise perception in a certain rythm However if information of the speech shall be brought about it is necessary to decode the electric signal and stimulate electrically the adequate nerve fibres which can conduct the signal to the auditory cortex for decoding it to meaning If that ever can be possible it shall probably be a long way to go

7. Summary

The history of electrophonic effect studies is reviewed from the very beginning by A Volta in 1800 up to the recent years' attempt to stimulate directly the hair cells and/or hearing nerve with electricity by means of implanted electrodes The evoked hearing sensation when a sinusoidal current is applied to the head using five different electrode systems is thoroughly examined and described The various kinds of hearing sensation elicited are dependent upon the type of electrode system used, upon the frequency and voltage of the electrical signal and upon the properties of the ear tested

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II Both normal and operated ears may perceive a tone The characteristic features of the tone i e the harmonic content the intensity the transient effect etc are a function of the electrode system in use

The different electrode systems are examined in details with respect to the location of the transducer and the possible transducing mechanisms

It is concluded that the transducing mechanisms involved in the tonal response are several and that their locations are peripheral to the cochlea In a series of analogue experiments to the electrode systems applied to humans the transducing mechanism in the various electrode systems are unveiled Besides well known transducing mechanisms as the Johnsen Rahbek effect the electrostatic transducing mechanism (vibrating electrodes and/or vibrating dielectric) electrocapillarity effect, two types of transducing mechanisms not known to have been described earlier in the litterature are accounted for

These are

1) A linear transducer, causing first harmonic sound by means of variation in adhesive forces modulating the sliding or rotating movement of the active electrode This is the main mechanism of the two involved in the fricative effect

2) A square law transducing mechanism bound to the primary shell of hydrated ions – or ions forming a shell with other types of polarized molecules – varying the packing under influence of a varying electric field thereby causing pressure variations in the liquid – i e an electrostriction phenomenon

The electrophonic effect eliciting a tonal sensation is therefore considered explained as the result of different transducing mechanisms in the different electrode systems all transducers located peripheral to the cochlea From the location of the transducer the vibrations are transmitted to the inner ear by means of bone, cartilage and air conduction The electro-anatomical properties of the cochlea and our recent knowledge of the cochlear microphonics strengthen the concept of peripherally located transducers

It is not considered of special advantage to use the electrophonic effect for diagnostic or therapeutic purposes

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The different electrode systems are examined in details with respect to the location of the transducer and the possible transducing mechanisms.

It is concluded that the transducing mechanisms involved in the tonal response are several and that their locations are peripheral to the cochlea. In a series of analogue experiments to the electrode systems applied to humans, the transducing mechanism in the various electrode systems are unveiled. Besides well known transducing mechanisms as the Johnsen Rahbek effect, the electrostatic transducing mechanism (vibrating electrodes and/or vibrating dielectric), electrocapillarity effect, two types of transducing mechanisms, not known to have been described earlier in the litterature, are accounted for.

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Acta

OTO-LARYNGOLOGICA

SUPPLEMENT 342

Histological Classification of Larynx and Hypopharynx Cancers and their Clinical Implications

Pathologic Aspects of 2052 Malignant Neoplasms Diagnosed at the ORL Department of Padua University from 1966 to 1976

BY

ALFIO FERLITO

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Monograph dedicated to

Professor Michele Arslan

upon his retirement as Director of the
Department of Otolaryngology of the
University of Padua School of Medicine

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Foreword

This monograph is the most important undertaking of one of my Collaborators, and represents the best evidence of the need for a constant co operation between the clinician, who must be a pathologist, and the pathologist, who must in turn be a clinician

This work is a basic part of an investigation still under way at our Department on the neoplastic disease located in particular in the larynx and hypopharynx Apart from an exhaustive illustration and description of common and rare malignant epithelial tumours of the larynx and hypopharynx, the present study lays stress on the fact that cancers of the larynx must be classified not only on the basis of the site and extent of the lesion, as recommended by the TNM system of tumour classification, but also by taking into account the histologic type, the histologic grading of malignancy and the tumour host relationship

The often unpredictable course of the neoplastic disease, the long standing discussions on whether surgery is indicated or not, and the advantages of the various techniques are no longer valid if the tumour is considered biologically in its entirety The malignant tumour is an intrinsically different entity from case to case, living parasitically on another living entity capable of counteracting tumour invasion Such resistance varies from person to person and in the same subject in different moments

I hope this work will be welcome and at the same time understood for its help in bringing to focus treatment of laryngeal neoplastic diseases, thus discarding commonplaces and resolving some controversial questions

The fact that this monograph is dedicated to our Master Prof Michele Arslan, is for us an opportunity for gratitude and honour, because the attainments of the present study are the result of our following the clinico-biological trend he has always supported in his activity and in the training of his Collaborators

Oscar Sala

I. Introduction

A. The "compartments" of the larynx: critical considerations

As is known, the larynx has a different embryological derivation for its compartments The supraglottic region develops from the bucco pharyngeal anlage, whereas the glottis and subglottis derives from the tracheobronchial anlage This different embryological origin has led to the conclusion that the larynx is made up by two hemilarynges superposed not only embryologically but also clinically, in particular in tumour pathology

A comprehensive investigation on laryngeal tumour diseases is being conducted at our department The results so far attained, based on objective anatomopathological and clinical elements, have convinced us that the subdivision of the larynx in three regions—supraglottic, glottic and subglottic—is acceptable only for descriptive purposes, but not from the point of view of tumour pathology Baclesse (1949) and later Lederman (1952) have made an attempt at classifying the laryngeal neoplasm as supra glottic, glottic and subglottic, but such a clear cut distinction does not always correspond, particularly in advanced cancers, to the actual extent of the neoplasm which cannot conceivably be assessed macroscopically

As a matter of fact, a laryngeal tumour may involve simultaneously the above three regions, though clinically it seems to be restricted to one or two of them Once the neoplasm has grown larger than the size of the initial lesion, its borders are hard to be established, not to speak of those tumours growing insidiously within the organ tissue (the so-called iceberg tumours")

Therefore in practice, a laryngeal cancer diagnosed as belonging to the supraglottic region, may well infiltrate the glottis, though this is not objectively observable This means that the fact of classifying an advanced cancer as belonging to one region or another is only indicative, and that the extent of a neoplasm cannot be adequately evaluated on the basis of a macroscopic examination Unlike it is commonly believed, a malignant neoplasm develops regardless of the so-called strong lines and weak lines mentioned by Bocca et al (1976) The invasion of the glottis and sometimes, though less frequently, of the subglottis, is not uncommon, particularly in case of ulcero-infiltrating cancers of the laryngeal ventricle Furthermore, Skolnik et al (1970) believe that the extent of advanced tumours of the larynx subjected to radiotherapy cannot be accurately assessed clinically

Tumours of the supraglottic region have been called "ascending cancers" because of their tendency to invade the pre-epiglottic area and the aryepiglottic folds According to Baclesse (1949) and Shaw & Epstein (1959) never do supraglottic tumours invade the glottis This opinion is also shared by Bocca et al (1968)

Histopathological studies by well known researchers (Szlęzak, 1966, Gunnel & Baerthold, 1967, Hommerich et al, 1971, Olofsson & van Nostrand, 1973, Olofsson, 1974, McDonald et al, 1976 and Micheau et al, 1976), which we also confirm, have demonstrated that supraglottic tumours may actually invade the glottic area and spread even further to the subglottis, though clinically the neoplasms appear to be restricted to the supraglottic region This statement is even more valid when considering tumours of the laryngeal ventricle

The concept of the embryological barrier and of the anatomical compartments delimiting neoplastic invasion is therefore no longer acceptable in the light of the above data As a result, the

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more malignant is the neoplastic lesion, the more atypical is tumour spread to the whole larynx

The different embryological origin of the laryngeal compartments may explain the histo genesis of some histological types of tumour that may be found only or mainly in one region or the other of the larynx For example, onco logically, the subglottis is the region of the larynx in which tumours histologically similar to those arising in the lung have been found

B Laryngeal and hypopharyngeal neoplasms anatomo-clinical considerations

Embryologically and anatomically, the larynx and hypopharynx are two distinct entities Neo plasms arising from these regions have a dif ferent biological behaviour, even though the histological type of tumour is the same In fact, everyone knows how uncertain the prognosis of hypopharyngeal tumours is, also when the neo plasm is well differentiated and limited in its extent In current practice, however, such a distinction is possible only for tumours at their initial stage, which remain relatively limited in extension

As a matter of fact, it is difficult to dissociate the neoplastic disease of the two organs since a tumour of the larynx or hypopharynx spreads beyond the anatomical limits of the organ in which it has originated and invades the adjacent organ, becoming an entity the course of which is more conditioned by general factors than by local anatomical factors

Another reason that has made us to consider the two neoplasms combinedly is the fact that many authors still use the term extrinsic cancer of the larynx to designate tumours arising in the pyriform sinus

Furthermore, from the pathomorphological point of view, the anatomical characteristics of the two regions are such as to allow a single histologic classification owing to the fact that in both neighbouring regions with indistinct and often arbitrary limits the same histological types of tumour may be seen, though one type usually prevails in one region or another of the larynx and hypopharynx

C. Biopathological factors for evaluation of laryngeal and hypopharyngeal cancer

The TNM system of tumours classification adopted by the International Union Against Cancer (UICC) and by the American Joint Committee for Cancer Staging and End Results Reporting (AJCCS) takes into consideration only the site and the extent of the tumour, this criterion is to be considered inadequate and fallacious for various reasons (Sala, 1976*a b*) However accurate, a clinico topographical ex amination of the tumour does not actually reveal the biological nature of the lesion

Daly (1967) strongly criticizes "all forms of clinical classification of cancer We quote from the author for a better understanding of his thought 'There are several problems connected with all classifications which need to be stated

1 They are based on definitions of anatomical sites and regions But carcinomas are no respecters of anatomical boundaries
2 Attempts at refinement of classification add new categories and wind up with insignificant numbers in each category
3 Anatomical boundaries are shifted to meet the convenience of reporting large numbers of cases, or to segregate the good from the poor results
4 Classification of a tumor will vary from one observer to another The degree of the varia tion has not been studied as in other clinical situations
5 The principal weakness of all classification is the lack of a way of a) measuring the malignancy of the tumor and b) evaluating the host tumor relationship'

We would like to add that laryngoscopy does not always allow to assess the extent of the tumour (iceberg cancers and intramural and submucosal cancers), as well as the presence or

absence of lymph nodes metastases cannot be detected with certainty by clinical palpation of the neck For this purpose we stress that experience has shown us that enlarged lymph nodes may be the expression either of cellular immune response (T-dependent) or of humoral immunity (B dependent) and be free from metastases Conversely, lymph nodes normal in size or even smaller may be metastatic Therefore, it must be emphasized once again that an enlarged lymph node must not be identified with a metastatic lymph node All that is an indication of how difficult it is to accurately assess the staging of a tumour disease

All the above limits largely invalidate the different clinical classifications of tumours proposed by UICC and by AJCCS, which employ the TNM system, as well as other classifications proposed by various authors (Norris, 1963, Tucker et al, 1971, Sessions & Ogura, 1974, Shepperd, 1974, etc)

The end result statistics are partly unreliable by the subjectivity conditioning the pathological diagnoses of dysplasia, diskeratosis, keratosis, keratotic squamous papilloma, carcinoma in situ, microinvasive carcinoma, verrucous squamous cell carcinoma (Batsakis, 1974, Ferlito, 1975*a*, *c*, Friedmann & Osborn, 1976) Another reason that makes all classifications arbitrary is the fact that malignant tumours are often generically referred to as "cancers", irrespective of their histological types

The neoplasm must be evaluated in its entirety, because it is an atypical biological entity expanding parasitically inside another biological entity, with a relationship varying from case to case

In our opinion, the factors to be considered are numerous (Sala & Ferlito, 1977) Here are the most important

1) site,
2) extent (which means not only the size of the primary neoplasm but also its nodal and visceral metastases),
3) the histologic type,
4) the histologic grading of malignancy, this parameter depends on a) the degree of structural differentiation, b) the frequency of mitoses, c) the cellular and nuclear pleomorphism, d) necrosis, and e) invasion,
5) immune response of the host

Factors of a lesser importance, which at times have been overestimated, are the age of the patient, his general condition, chronic infectious diseases—tuberculosis and syphilis seem to favour the development of a tumour—the association with other tumours, in particular of the lung (Sala & Ferlito, 1975, Ferlito & Di Bonito, 1976), etc Actually, these factors reflect upon the immune system

II. Aim of the Study

The present paper aims at underlining the variety of histological types of tumours and their importance with regard to prognosis in apparently uniform organs such as the larynx and hypopharynx The correct microscopic identification of a neoplasm must help the clinician working jointly with the pathologist to adopt the most adequate therapeutic programme, excluding those treatments which might not only be of no use but altogether harmful The most typical example is the contraindication of radiation therapy in case of verrucous squamous cell carcinoma of the larynx (Kraus & Perez-Mesa, 1966, Biller et al , 1971, van Nostrand & Olofsson, 1972, Ogura & Biller, 1973, Bauer, 1974, Ferlito, 1975*b*, Hyams, 1975*b*, Ferlito et al , 1976)

The importance of the histological type of tumour has long been known, but is generally limited to the most common, well-known histological types Distinction is currently made between squamous carcinoma and anaplastic carcinoma

Laryngeal and hypopharyngeal cancer—a generic term to indicate any malignant neo-

Table I *2052 primary and recurrent malignant neoplasms of the larynx and hypopharynx diagnosed at the ORL Department of Padua University for an 11-year period from Jan 1, 1966 to Dec 31, 1976, after re-classification*

Histological type[a]	No of cases		Percentage
	Prim	Recurr	(primary neoplasms only)
Carcinoma in situ	54		2 81
Squamous cell carcinoma	1 605	110	83 51
Verrucous squamous cell carcinoma	71	4	3 70
Spindle-cell carcinoma	12	3	0 63
Lymphoepithelial carcinoma	1		0 05
Undifferentiated carcinoma	139	5	7 24
Oat cell carcinoma	1		0 05
Carcinoid	1		0 05
Adenocarcinoma	7		0 36
Giant cell carcinoma	1		0 05
Clear cell carcinoma	1		0 05
Adenosquamous carcinoma	8	3	0 42
Mucoepidermoid carcinoma	10	1	0 52
Adenoid cystic carcinoma	1		0 05
Acin cell carcinoma	1	1	0 05
Lymphoma	5		0 26
Malignant fibrous histiocytoma	2	2	0 10
Fibrosarcoma	1		0 05
Pleomorphic liposarcoma	1	1	0 05
Total	1 922	130	100 00

[a] Most of the terms adopted to designate the neoplasms are those advised by the Committee on Tumour Nomenclature of the International Union Against Cancer Springer Verlag Berlin 1969

plasm, either epithelial or of connective origin, or mixed, though in the majority of cases it is a squamous cell carcinoma—may display different aspects according to the cell type from which it originates (stratified columnar epithelium, glandular epithelium, Kultschitsky cells, etc) and to their histologic grade of differentiation

Current terminology is often arbitrary and above all inaccurate Incidentally, it must be said that the basiloid carcinoma does not exist in the larynx, though many authors have mistakenly included it in their series A careful review of those tumours labelled as basiloid carcinoma or transitional cell carcinoma reveals that they actually are poorly differentiated squamous carcinomas (Bauer, 1974) Michaels (1976) does not accept the term "transitional carcinoma" and is of the opinion that such a definition alters the scientific appraisal of the tumour

The number of malignant neoplasms of the larynx and hypopharynx diagnosticated at the Department of Otolaryngology of Padua University has always been very high and reaches about two hundred cases a year

In Table I are reported all malignant neoplasms of the larynx and hypopharynx and recurrences encountered from January 1966 to the end of December 1976 at the ORL Department of Padua University

Starting from November 1974, *the systematic study of the surgical specimen* (primary lesion and regional lymph nodes) has become a routine practice at our Department Such an investigation has enabled us to have a clear, comprehensive bio-pathological view of the behaviour of each case of tumour diseases—a behaviour which is different from patient to patient

The results attained with this systematic study have induced us to start an accurate revision of all histological preparations from the previous years available at the Department This work of revision will be completed with the long term outcome The results concerning a first group of 104 patients (cases treated in 1968) with laryngeal and hypopharyngeal tumours revised histopathologically and checked as to long-term survival, have already been communicated to the Collegium O R L A S in September 1975 (Sala & Ferlito, 1976)

Particular stains had to be prepared from the blocks, as some histological types of tumour needed a more accurate identification on the basis of the newest criteria of nosological classification of neoplasms

All histological sections have been accurately reviewed and *classified or re-classified* by the pathologist of the Department (A Ferlito) according to the most up to date principles for histologic classification of tumours and evaluation of the histologic grading of malignancy, as well as on the basis of the tumour host reaction, which conditions the clinical course of the disease

This work of revision has made it possible to recognize some rare histological types of tumour which had been first identified only in recent years, and to achieve a more ample insight of the neoplastic disease Thus, it appears to us that the histological response from the pathologist to the clinician should take the form of a table showing all the characteristics distinguishing the tumour disease Of course, such table needs to be easily understood by the clinician and evaluated in its practical therapeutic significance (see Discussion and Conclusions)

The main purpose of the present investigation is to bring to focus the different histological types of laryngeal and hypopharyngeal malignant tumours

As said before, in order to better define the characteristics of malignancy of the tumour, consideration must be given not only to the histological type but also to the histologic grading of malignancy and the tumour-host relationship By taking the above three factors into account, the pathologist is able to tell the clinician the degree of malignancy of the lesion In some cases, these three factors are strictly connected and consequential For example, the verrucous squamous cell carcinoma is a tumour of low-grade malignancy and a marked cellular response

(Ferlito, 1975*b*) whereas the anaplastic carcinoma has a high histological grading of malignancy and a poor tumour-host reaction Prognosis is therefore completely different for the two cases which may be considered as extremes—it is excellent in the first case and poor in the second

Again, these factors (histologic type, histologic grading of malignancy and cellular response) may show a dissociated behaviour, varying from patient to patient and also in the same subject at different moments

As a result, establishing a correct diagnosis is of the utmost importance The biopsy specimen must be "significant", that is, it must be representative of the tumour structure Therefore, a reasonably large, deep sample of the tissue must be removed by means of incision and not just from the surface of the tumour This allows the pathologist to evaluate the lesion in all its characteristics and to formulate a more accurate diagnosis than simply that of cancer At operation, the surgeon should take into consideration that the specimen removed must be fixed, imbedded and cut, and then the sections stained, therefore if the fragment is too small, the pathologist might meet more difficulties in understanding the lesion correctly

III. Histological Classification of the Larynx and Hypopharynx Cancers

A critical review of biopsy specimens and the systematic study of surgical specimens (larynx and lymph nodes) has convinced us that the larynx also exhibits a *great histological variety* of tumours The histological type is of the greatest importance when establishing treatment and it also conditions the long term prognosis

The proposed classification is the result of the every-day experience in diagnosing laryngeal and hypopharyngeal cancer, and of the recent developments from the world literature on the subject The present classification concerns only the *malignant epithelial tumours*, leaving out for the time being a classification of tumours arising in connective tissue which, as is known, are exceptional in the larynx and hypopharynx

This classification is mainly based on pathomorphological criteria, and right from now we say that this attempt is by no means complete and definite Existing classifications of laryngeal and hypopharyngeal cancers are often incomplete or simplistic, or are included in those concerning cancer of the upper respiratory tract

As it is not possible to outline a classification of tumours at the same time simple and comprehensive of all varieties of laryngeal and hypopharyngeal cancers that may be encountered, we think that the most complete classification, though perfectible, might be as shown in Table II

Table II *Histological classification of laryngeal and hypopharyngeal cancers*

1 Carcinoma in situ
2 Squamous cell carcinoma
  2*a* Well-differentiated (grade I)
  2*b* Moderately well-differentiated (grade II)
  2*c* Poorly differentiated (grade III)

  Variants of squamous cell carcinoma
  2 1 Verrucous squamous cell carcinoma
  2 2 Spindle-cell carcinoma
  2 3 Lymphoepithelial carcinoma
3 Undifferentiated carcinoma
4 Oat cell carcinoma
5 Carcinoid
6 Adenocarcinoma in situ
7 Adenocarcinoma (NOS)[a]
8 Giant cell carcinoma
9 Clear cell carcinoma
10 Adenosquamous carcinoma
11 Malignant mixed carcinoma
12 Mucoepidermoid carcinoma
13 Adenoid cystic carcinoma
14 Acin cell carcinoma
15 Carcinosarcoma
16 Unclassified carcinoma

[a] Non otherwise specified

1 Carcinoma in situ[1] (or intraepithelial carcinoma)

The tumour, also called non invasive or pre invasive carcinoma, is characterized by cells atypical in shape, size and nucleus, which appear hyperchromatic Mitoses may be numerous The basement membrane is not involved and the subepithelial tissue commonly shows chronic inflammation It is a tumour displaying all criteria of malignancy except invasiveness The changes of carcinoma in situ may sometimes be found near an invasive squamous cell carcinoma Almost always, these neoplastic foci are "in situ" carcinoma originating from squamous cells, at times, however, undifferentiated aspects

[1] Most of the terms adopted to designate the neoplasms are those advised by the Committee on Tumour Nomenclature of the International Union Against Cancer Springer Verlag, Berlin 1969

may be seen Carcinoma in situ, which may be multicentric in origin, may co exist with intraepithelial carcinomatous changes of the epithelium of the mucous glands and their ducts In these cases the term "intramucosal carcinoma" rather than that of "intraepithelial carcinoma" defines better the actual extent of the disease Differential diagnosis must be made from epithelial dysplasia, the lines of demarcation between the two lesions being often vague and uncertain

Often this lesion is a "false" carcinoma "in situ", inasmuch as a small biopsy fragment allows only the identification of an apparent carcinoma "in situ" Another biopsy of adequate size or the surgical specimen not rarely do confirm that the lesion previously said to be "carcinoma in situ" displays in adjacent areas tumour infiltration of the basement membrane and stroma, and therefore the lesion is a true invasive carcinoma It appears therefore that a diagnosis of carcinoma "in situ" established only from examination of small biopsy fragments cannot be considered as reliable and taken without reserve (Ferlito, 1975*a*) Bauer & McGavran (1972) have found that 75 percent of invasive carcinomas were associated with carcinoma in situ All that does not deny the existence of true isolated carcinomas "in situ" which, on the other hand, must be considered as a malignant and not a pre malignant lesion

Both surgery (in my opinion preferable, particularly in young patients) and radiation therapy may be adopted with good results

2 Squamous cell carcinoma (or epidermoid carcinoma)

This is the most common laryngeal malignant tumour, it originates from surface epithelium—both from stratified squamous epithelium and from pseudo-stratified ciliated columnar epithelium—after this has undergone phenomena of squamous metaplasia The following distinction is made, according to the degree of tumour cell differentiation, taking into account that the tumour must be graded according to the most dedifferentiated area found

Table III *Keratinizing carcinoma larynx (excluding true cord lesions)*

Differentiation	Cases	Lymph nodes metastases No	Lymph nodes metastases %	Dead of cancer (10 years) No	Dead of cancer (10 years) %
Well and moderate	62	12	19	14	23
Poor	38	20	63	15	40
Total	100	32		29	

From Washington University Medical Center

2 a *Well differentiated* (grade I) it is characterized by polygonal or prickle-type cells, stratification, intercellular bridges and epithelial pearls Nuclei are hyperchromatic and the nuclear-cytoplasmic ratio is altered The connective stroma is always haphazardly infiltrated by irregular nests or strands of malignant epithelial cells The mitotic figures are rare

2 b *Moderately well-differentiated* (grade II) it is characterized by polygonal or prickle type cells, stratification intercellular bridge formation Epithelial pearls are not present Mitoses are numerous and atypical Nuclei are pleomorphic

2 c *Poorly differentiated* (grade III) the tumour is composed of undifferentiated cells showing nuclear pleomorphism and hyperchromasia, and little but distinct evidence of intercellular bridge formation

Clinically, there is a correlation between cellular differentiation and the incidence of cervical lymph nodes metastases In fact, poorly differentiated carcinomas show greater aggressiveness than well differentiated and moderately well differentiated neoplasms

This fact has been observed and accurately reported by McGavran et al (1961), who examined the incidence of cervical lymph nodes metastases from 96 squamous cell carcinomas

Table IV *Relationship of histologic grade of squamous cell carcinoma of the larynx and survival*

Grade	No of cases	Survival				
		1 year	2 years	3 years	4 years	5 years
I	29	26 89 66 %	24 82 76 %	24 82 76 %	24 82 76 %	24 82.76 %
II	55	39 70 91 %	35 63 63 %	34 61 81 %	31 56 36 %	30 54 54 %
III	20	9 45 %	7 35 %	7 35 %	4 20 %	4 20 %
All patients	104	74 71 15 %	66 63 46 %	65 62.50 %	59 56 63 %	58 55 77 %

of the larynx and noted that the poorly differentiated carcinomas showed a high incidence of metastases (49 %), whereas moderately well-differentiated neoplasms had a metastatic incidence of 22 % and well differentiated tumours metastasized less frequently (11 %)

In Table III, some data of practical interest are reported (Bauer, 1974)

Also recent investigations carried out at our department by Sala & Ferlito (1976) have proven that a correlation exists between the degree of cellular differentiation of squamous cell carcinoma and survival Table IV and Diagram 1 show some significant results of a comprehensive analysis made on 104 patients with carcinoma of the larynx

2 1 *Verrucous squamous cell carcinoma* (or verrucous carcinoma)

It is a cancer of low-grade malignancy and no metastatic spread It is one of the three variants of squamous cell carcinoma Histologically, it is composed of islands and solid or papillomatous cords of highly differentiated epithelial squamous cells Mitoses are rare Cytologic criteria of malignancy are lacking The stroma discloses numerous inflammatory cells, mainly lymphocytes and plasma cells tending to delimit the tumour mass Epithelial pearls and small cysts may be observed These foreign body granulomas, included in a granulomatous tissue, are often seen next to epithelial pearls or keratinized matter This tumour has been described in the larynx by several authors (Kraus & Perez Mesa, 1966, Biller et al, 1971, van Nostrand & Olofsson, 1972, Demian et al, 1973, Elliott et al, 1973, Babighian & Ferlito, 1974, Bak & Erdős, 1975, Biller & Bergman, 1975, Ferlito, 1975*b*, Fisher, 1975, Rider, 1975, Burns et al, 1976, Ferlito et al, 1976)

Verrucous squamous cell carcinoma is an enigmatic neoplasm causing remarkable difficulty both to clinician and pathologist

The histologic criteria for identifying the tumour are the following

1) exophytic, warty tumour with multiform filiform like projections,
2) the tumour must show greatly thickened,

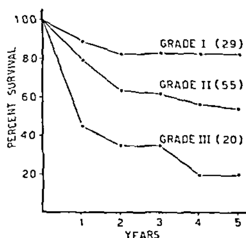


Diagram 1 Survival of patients with cancer of larynx as related to histologic grade of the tumour ( ) = number of patients

club-shaped, papillomatous folds of well differentiated keratinizing squamous epithelium,
3) absence of easily recognizable cytologic abnormalities usually associated with malignant squamous lesions,
4) the advancing margin of the tumour must be of a "pushing" rather than an infiltrating type,
5) the advancing margin of the tumour must be associated with considerable inflammatory response in the adjacent tissue

It is a cancer not easy to be identified, with low-grade malignancy and with distinctive clinical evolution It shows poor local invasion and no evidence of regional or distant metastases (Biller et al , 1971)

Surgery is the elective treatment, but since the tumour may easily recur, ample excision is required Radiation therapy should not be employed, since it may alter the nature of the neoplasm to a highly malignant, by inducing anaplasia or causing a sarcomatous transformation of the tumour and consequently a rapid metastatic dissemination The failure of radiotherapy seems to be connected with the [illegible] that by causing lymphocytotoxic effects, this treatment makes lymphocytes and selectively small lymphocytes (T-lymphocytes) decrease (Ferlito et al , 1976) As a result, there is an impaired cellular immunity which reflects on the host's response to tumour (Burge, 1975, Dubois et al , 1975) Rider (1975), on the contrary, maintains that the results of good radiotherapy are at least equal to surgery and much better functionally Burns et al (1976) are of the opinion that both surgery and radiotherapy may be adopted successfully in the management of the lesion In their opinion conservation surgery is more indicated in case of small verrucous tumours, whereas radiotherapy should be elected for tumours sufficiently large to require total laryngectomy if treated surgically Prophylactic radical neck dissection is not indicated (van Nostrand & Olofsson, 1972, Ogura & Biller, 1973 Thomas et al , 1973, Biller & Bergman, 1975 Ferlito 1975*b*)

2 2 *Spindle cell carcinoma* (or pseudosarcoma)

It is often mistaken for carcinosarcoma or sarcoma (in particular fibrosarcoma) because numerous cells of the tumour are spindle shaped It is on the contrary an uncommon variant of squamous cell carcinoma, which is also mistakenly called collision tumour, or carcinosarcoma or pseudosarcoma associated with squamous cell carcinoma, or pseudosarcoma, or squamous cell carcinoma with sarcoma-like stroma, or pleomorphic carcinoma, or pleomorphic pseudosarcomatous carcinoma, or spindle cell variant of squamous cell carcinoma As a result, some reservations must be made for many a case of laryngeal sarcomas previously reported in the literature This tumour is most often found in the true vocal cord

It is characterized by the presence of atypical, often bizarre cells with pleomorphic nuclei Mitoses are atypical Occasional multinucleated cells are present and the surface epithelium is often ulcerated An accurate histological examination always discloses areas of more or less differentiated squamous cell carcinoma, next to apparently sarcomatous areas The malignancy of the sarcomatous elements has not been proved with certainty The absence of the epithelial component, which may well be lacking in small biopsy fragments, may lead to the wrong diagnosis of sarcoma Goellner et al (1973), who reviewed cases of pseudosarcoma of the larynx at the Mayo Clinic, state that the pseudosarcomatous stroma is a reactive proliferation of histiocytes and fibroblasts, suggesting a response to the squamous carcinoma, as revealed by light and electron microscopic features and enzyme histochemical findings Battifora (1976), who has recently carried out transmission electron microscopy studies of two cases of spindle cell carcinoma, suggests that the pseudosarcomatous components of the tumour originates from mesenchymal metaplasia of squamous cells and that collagen is synthetized by these metaplastic cells This tumour very likely originates from surface epithelium

Macroscopically, the tumour may be either exophytic—polypoid or pedunculated—or sessile and infiltrative, the prognosis being considered to be better in the former case and poorer in the latter (Sherwin et al, 1963, Appelman & Oberman, 1965) Batsakis (1974) state that if the tumour is of the pedunculated type, local excision followed by radiation therapy has given excellent results as to 5 year survival rate Randall et al (1975) have recently studied nine cases of spindle cell carcinoma of the larynx reporting also follow up, and state that a correlation between gross morphology and prognosis cannot be established, whereas the prognosis seems to be more closely related to the size and location of the tumour Ferlito & Babighian (1972) have evidenced a case of pseudosarcoma (spindle cell carcinoma) of the epiglottis (encountered at the Department of Pathologic Anatomy of the University of Trieste), in which in spite of the polypoid aspect of the lesion, the neoplasm had spread metastases of the squamous type to cervical lymph nodes, cranium, scapulae, pelvis, upper third of femuri and some vertebrae, as seen at autopsy Therefore, it is no longer justified to believe that a polypoid spindle cell carcinoma has a better prognosis than the sessile infiltrative variety Hyams (1975*a*) upon examination of the files of the Registry of Otolaryngic Pathology of the Armed Forces Institute of Pathology from 1960 through 1970, reported a clinico pathologic correlation of 39 cases of spindle cell carcinoma of the larynx, and though tumours were usually of the prominent polypoid type the follow-up information on 20 cases indicated a two years mortality of 40%, refuting the contention that this entity is of low grade malignancy

In conclusion in agreement with Friedel et al (1976) but contrarily to Appelman & Oberman's (1965) and Andrews & Oliver's (1972) statements, the prognosis of these neoplasms is poor as they not infrequently metastasize

So far there are no valid reasons to make a choice between surgery and irradiation, since in the half of cases treated by either forms of treatment there have been local recurrences Randall et al (1975) recommend surgery till the role and value of radiotherapy will be better assessed Friedel et al (1976) recommend that they be treated aggressively like any other carcinoma of the head and neck

This tumour has been described in the larynx by many authors, who labelled the neoplasm with different terms (Szmurlo, 1894, Kahler, 1908, Ullmann, 1922, Consiglio, 1931, Moulonguet & Leroux Robert, 1933, Ricci, 1933, Baker & Lambert, 1934, Schwarz, 1936, Aubry & Leroux Robert, 1937, Frank & Lev, 1940, Wessely, 1940, Moore, 1951, Coassolo, 1952, De Vido, 1953, Withalm, 1953, Herail, 1954, Leyrit, 1954, Fior, 1955, Griepentrog, 1955, Lane, 1957, Baker, 1959, Invernizzi, 1959, Fini Storchi, 1960, Pico, 1960 Grigg et al, 1961, Kratz & Ritterhoff, 1961, Norris & Peale, 1961, Boccuzzi, 1962, Legier & Vancouver, 1962, Walter, 1962, Kaneko, 1963, Sherwin et al, 1963, Appelman & Oberman, 1965, Ascenzi & Scalori, 1965, Rucco & Zerneri, 1965, Ardouin et al, 1966, De Brux et al, 1966, Filotico & Trabucco, 1966, Gerard Marchant et al, 1966, Jobard et al, 1966, Colafranceschi & Di Filippo, 1967, Vengerowskij & Filatov, 1967, Grynsztajn & Kurnatowski, 1968, Himalstein & Humphrey, 1968, Perilli, 1968, Pohl, 1968, Taniewski & Czerwinska, 1968, Verhest & Jortay, 1968, Davies, 1969, Kubo et al, 1969, Leonardelli & Pizzetti, 1970, Lesoine & Jopp, 1970 Lichtiger et al, 1970, Usmanova, 1970, Galle et al, 1971, Staley et al, 1971, Andrews & Oliver, 1972, Cachin et al, 1972, Escalona Zapata, 1972, Ferlito & Babighian, 1972, Schloss & Novick, 1972, Goellner et al, 1973, Cortes & Gil, 1974, Koudim & Karam, 1974, Kupper & Blessing, 1974, Vincent et al, 1974, Hyams, 1975*a*, Miller, 1975, Randall et al, 1975, Schmidt Baümler & Rupp, 1975, Friedel et al, 1976)

2 3 *Lymphoepithelial carcinoma* (or lymphoepithelioma)

It is characterized by the presence of poorly differentiated cells with indistinct cell borders

arranged in a mosaic pattern and intermingled with masses of lymphocytes admixed with epithelial cells The lymphocytes are not a neoplastic component of the tumour Atypical epithelial cells are rounded or polygonal and large in size, with clear cytoplasm and tend to form syncytial appearances The nucleus is oval and vesicular, with chromatin located peripherally The nucleolus is large and prominent Mitoses usually are conspicuous and the connective stroma is nearly always scanty Though widely used, the term lymphoepithelioma is a misnomer, since the tumour is actually a variant of squamous cell carcinoma In fact, electron microscopy has demonstrated that lymphoepithelioma has tonofilaments, desmosomes and keratin fibrils, and this indicates that the tumour originates from epithelium (Mori & Lennert, 1969)

This tumour has been found in nasopharynx, nasal fossae, maxillary sinus, tonsil, posterior third of the tongue, oropharyngeal region and thymus, but may also arise in the larynx (Micheau et al, 1976), often in the laryngeal ventricle at the site of the laryngeal tonsil where clusters of lymphocytes are present, and in the hypopharynx, where nests of lymphocytes may be found Dockerty et al (1968) reported a case encountered at the Mayo Clinic, of a lymphoepithelioma of the hypopharynx in a 60-year old man, who died for metastatic dissemination 10 months after he had been treated with roentgen raytherapy Autopsy did not evidence a neoplastic lesion at the site of the primary tumour, which appeared free from the disease, whereas widespread visceral and nodal metastases were present

I have also encountered a case of lymphoepithelioma in a 60-year old male patient who showed cervical lymph nodes metastases He underwent surg[illegible] followed by radiotherapy, but died ten mo[illegible]s after diagnosis was made for widespread [illegible]mour dissemination The neoplasm was loc[illegible]ed in the left pyriform sinus For further detai[illegible]s [illegible]ee Ferlito 1976*e*

In our opinion [illegible] is useful to distinguish this tumour and designate it as lymphoepithelial carcinoma, because it has distinct clinical and pathological characteristics that allow a distinction both from squamous cell carcinoma and undifferentiated carcinoma Differential diagnosis must also be made from histiocytic lymphoma The neoplasm, though radiosensitive, is hardly radiocurable

3 Undifferentiated carcinoma (or anaplastic carcinoma)

This is surely an uncommon tumour but by no means an extremely rare one It is composed of anaplastic cells from the surface epithelium which haphazardly infiltrate the stroma Cells are arranged in clusters and nests, and display atypism and anaplasia Cell teratism as well as atypical mitoses are frequent The cytoplasm is indistinct and the nucleus is vesicular and hyperchromatic Areas of necrosis are often seen This tumour is also called *transitional cell carcinoma*, or *basiloid carcinoma*, a terminology which should be abandoned because it is improper and causes confusion

In our opinion the so called basiloid carcinoma of the larynx, the prognosis of which is considered to be less favourable than that of the common squamous carcinoma (Pietrantoni & Fior, 1958), does not occur in the laryngo-hypopharyngeal region Actually, the so-called basiloid carcinoma described by various authors (Orton, 1938, Eggston & Wolff, 1947, Caliceti, 1951—an incidence of 19 7% in his series', Withalm, 1953, Ash & Raum, 1956, Pietrantoni & Fior, 1958, Ash et al, 1964, Leonardelli, 1974, Motta & Bolognesi, 1976) are but undifferentiated or anaplastic carcinomas and as such they have a high biologic malignancy and a tendency to metastasize to lymph nodes The neoplastic cells, of a basiloid aspect, are therefore atypical undifferentiated cells of the pseudo-basiloid type These tumours are most often found in the laryngeal ventricle and in the false vocal cords

Anaplastic carcinoma is a highly malignant epithelial tumour and its prognosis is poor The lesion requires as radical as possible a treatment Surgery must not only eradicate the primary neoplasm, but also remove the cervical

lymph nodes bilaterally The poor cellular differentiation of this histological type of tumour greatly conditions the prognosis, because of the easy metastatic spread and recurrence if excision is not adequate The aspect of anaplastic or undifferentiated carcinoma may be present from the beginning or may be caused by radiation therapy

4 **Oat cell carcinoma** (or anaplastic small cell carcinoma)

The tumour is composed of numerous irregular clumps of elongated small cells with scanty cytoplasm and relatively large, hyperchromatic, oval, round or spindle-shaped nuclei, with delicate chromatin and an often visible nucleolus Mitoses are numerous The tumour cells are supported by a little vascular, fibrous stroma The cells may be arranged in solid masses and occasionally appear clustered around alveolar-like spaces Areas of necrosis are present This neoplasm may be mistaken for malignant lymphoma owing to the presence of roundish or oval cells with scanty cytoplasm which may resemble lymphocytes Most probably, the tumour originates from Kultschitsky cells and is histogenetically related to the APUD-System

Recently, a few cases of primary oat cell carcinoma of the larynx have been described (Olofsson & van Nostrand, 1972, Ferlito, 1974,[1] Benisch et al, 1975, Gelot et al, 1975, Mířejovsky & Hroboň, 1975) In ample series of tumours of minor salivary gland origin, Koss et al (1972) and Spiro et al (1973) mention oat cell carcinoma of the larynx as being akin to oat cell carcinoma of the bronchus In my opinion, their case was one of anaplastic carcinoma (it is a single case reported twice and separately in two ample series of tumours) Mířejovsky & Hroboň (1975) described a primary small cell carcinoma of the laryngeal ventricle with metastases to the liver Electron microscopy showed that the neoplasm had endocrine granules which were not argyrophilic

The clinical course of oat cell carcinoma of the larynx is rapidly fatal, but, fortunately, this is an extremely rare neoplasm Of the cases so far reported in the literature of a laryngeal occurrence of such tumour, all patients died a few months later, whereas only one was living two and one half years after diagnosis (the case described by Olofsson and van Nostrand, 1972)

5. **Carcinoid** (or argentaffinoma)

The tumour is composed of small uniform cells with round nuclei and poorly delineated cytoplasm, arranged in sheets or in nests of more or less uniform size surrounded by vascular strands of connective tissue The mitotic figures are very scanty Carcinoid belongs to the group of "apudomas", histogenetically deriving from the so called APUD System

A case of malignant carcinoid of the epiglottis, with neoplastic infiltration of the base of the tongue and bilateral lymph nodes metastases has been described (Goldman et al, 1969) The patient died after two years following discovery of primary tumour A few years later, Horikawa (1972) described a case of carcinoid tumour of the epiglottis Ackerman & Rosai (1974) have evidenced some cases of such tumour It seems that this tumour originates from Kultschitsky or enterochromaffin cells

The present writer has evidenced a carcinoid of the malignant variety located in the subglottic region Microscopically, the neoplasm had a frankly malignant aspect and appeared to infiltrate the right thyroid cartilage and the crico-thyroid membrane The female patient died about two years after right hemilaryngectomy and ipsilateral hemithyroidectomy The tumour had metastasized to the bones

The nonappendiceal origin carcinoid tumours are malignant and recommended treatment is wide surgical excision Goldman et al (1969) say

[1] This case has not been included in the present series as it had been encountered at the Verona Section of the ORL Department of Padua University

The one reported in Table I, unpublished has been evidenced in Padua.

that radiation therapy is non effective in the treatment of laryngeal carcinoid In fact, irradiation is not recommended as treatment for carcinoids, appendiceal and nonappendiceal

6. Adenocarcinoma in situ

This is a pre-invasive carcinoma involving the mucous glands It is characterized by the presence of epithelial cells atypical in shape, size and nuclear characteristics The nuclear-cytoplasmic ratio is altered The neoplasm never spreads *beyond the basement membrane which appears intact*

It is a rare lesion which may be a precursor of invasive adenocarcinoma It is often associated with carcinoma in situ, as reported by Brighton & Altmann (1942), Altmann et al (1952), Stout (1953) and Bridger & Nassar (1971) It may also be seen concomitantly with adenosquamous carcinoma as observed by Gerughty et al (1968) and Ferlito (1976*b*) It is probably a tumour of multicentric origin, showing many aspects in common with lobular carcinoma of the breast The treatment of choice is ample excision of the tumour, which fact not infrequently implies total laryngectomy

7. Adenocarcinoma (non otherwise specified)

The tumour is composed of atypical columnar epithelial cells arranged so as to form glandular structures The neoplasm originates from mucus-secreting glands Several cases have been reported in the literature (Solis-Cohen, 1892, Havens & Parkhill 1941 New & Erich, 1941 Eggston & Wolff 1947 McDonald & Havens, 1948, Toomey, 1967 Cady et al 1968, Adams & Duvall, 1971, Dogra 1973 Komorn et al, 1973, Whicker et al 1974 Fechner, 1975, Murray et al, 1975, Sessions et al 1975 Houle et al, 1976, Spiro et al 1976)

Some squamous cell carcinomas may sometimes display a glandular pattern though they are not adenocarcinomas

The prognosis is poorer than that of the commoner squamous cell carcinoma This is a radioresistant tumour, therefore wide surgical resection seems to be the best hope of cure (Whicker et al, 1974, Eschwege et al, 1975)

In one of our patients, an adenocarcinoma associated with squamous cell carcinoma of the larynx and cervical lymph nodes metastases has been observed Adenocarcinomatous aspects only were visible in nodal metastases

8 Giant cell carcinoma (or anaplastic giant cell adenocarcinoma)

The tumour is basically composed of numerous pleomorphic multinucleated giant cells, often with strongly acidophilic vacuolated cytoplasm, supported by a delicate fibrovascular stroma Tumour cells are sometimes extremely bizarre and resemble sarcomatous giant cells, but never do they exhibit striation (Heidenhain haematoxylin stain does not make cross-striation evident) *Numerous atypical mitoses are present* Multinucleated cells usually show many nuclei, but only rarely do they have an exceptional number of nuclei Multinucleated cells display phagocytic properties These cells are sometimes isolated, while in other areas they are arranged in small clusters At times, they are clustered around alveolar spaces (Gomori's stain evidences reticular fibres tending to form alveolar spaces) In many areas glandular like patterns may be noted Multinucleated cells vary in shape—they appear to be polyhedral, roundish, irregularly oval—and have abundant acydophilic cytoplasm Well delimited, hyperchromatic atypical nuclei in multinucleated cells often appear arranged in a cluster Chromatin is irregularly distributed PAS positive granules are present *in the cytoplasm of some giant cells* The tumour also shows highly atypical or frankly monstrous spindle cells with strongly hyperchromatic nuclei, resembling atypical cells of rhabdomyosarcoma This tumour has to be distinguished from squamous and anaplastic carcinoma in which giant cells have appeared as a consequence of radiotherapy

This neoplasm is exceptional in the larynx

Only one case has been described recently by the present writer (Ferlito, 1976*a*) who thinks that the tumour is of glandular origin

9. Clear cell carcinoma
(or large cell carcinoma, clear cell type)

The neoplasm is extremely rare in the larynx It shows the same morphological features as those of the clear cell carcinoma of the lung, the incidence of which is about 2–3 % of all pulmonary cancers It is composed of nests or clusters of clear, large rounded cells with non-staining cytoplasm and small vesicular central nuclei The cytoplasm is abundant and appears "empty" The cells may or may not contain glycogen Highly atypical, bizarre multinucleated cells may be noted The tumour is basically made up by solid masses of clear cells bounded by fibrous tissue At times, central vacuolation of the neoplastic masses may be seen Areas of necrosis may also be present There is no evidence of squamous or glandular differentiation, though a thorough examination of the whole laryngeal specimen may disclose abortive glandular differentiation and the presence of PAS positive matter in the lumen of glandular like structures Most probably this is a poorly differentiated adenocarcinoma of ductal origin (occasional tubular structures among the solid neoplastic masses may be noted) Differential diagnosis must be made from squamous cell carcinoma with large clear cells with hydropic cytoplasm and from metastases originating from clear cell carcinoma of the kidney

In our ample series, only one case of clear cell carcinoma of the larynx with lymph nodes metastases has been found The neoplasm had spread to the whole organ

The clear cell carcinoma and the giant cell carcinoma belong to the group of the large cell carcinoma, as seen in the lung

10 Adenosquamous carcinoma (or mixed adenosquamous carcinoma)

The tumour is composed of an admixture of adenocarcinoma with squamous cell carcinoma Commingled to the latter patterns are areas of undifferentiated carcinoma or so-called "basiloid" carcinoma Epithelial cells appear to be atypical in shape, size and nuclear characteristics, as well as in the nuclear-cytoplasmic ratio The tumour seems to originate from glandular and ductal epithelium, regardless of the fact that tumour cells are of the squamous, or basiloid or undifferentiated type and are arranged so as to form tubular, alveolar or ductal configurations Duct-like structures with intraductal epithelial proliferation may be seen The connective stroma is normal, though at times it may appear slightly myxoid Areas of "in situ" ductal carcinoma showing neoplastic cells of the anaplastic variety or of the squamous variety may also be noted A characteristic feature, though not a constant one, is the presence of large nests and solid masses composed of "glassy" cells bounded by fibrous tissue Their abundant cytoplasm is clear, PAS-negative and contains a prominent hyperchromatic nucleus, sometimes located peripherally Multinucleated cells may be seen Within the compact islands of glassy cells, masses of keratin and nests of atypical squamous cells are noted Histochemical methods (mucicarmine, PAS and DPAS) also evidence cells containing intracellular mucin in their cytoplasm Mucicarmino- and PAS-positive matter is also present in the lumen of some glandular structures The surface epithelium may appear intact or frankly carcinomatous, but infiltration always originates from glandular epithelium

This neoplasm might have a glandular origin or perhaps derives from ductal epithelium In lymph nodes metastases, adenocarcinomatous elements admixed with squamous elements are present

The tumour—which has also been called "glassy cell carcinoma" by Littman et al (1976), who found it in the uterine cervix—is clinically extremely aggressive and highly malignant and shows minimal or no cellular immune response in tumour stroma

Differential diagnosis must be made from mucoepidermoid carcinoma and adenoid-cystic carcinoma, but is not easy to be established

The therapy of choice appears to be radical surgery since the tumour is radioresistant (Leroux-Robert & De Brux, 1976)

Gerughty et al (1968) reported three cases of laryngeal occurrence of such neoplasm Four other cases have been recently described by the present writer (Ferlito, 1976*b*)

11. Malignant mixed carcinoma
(or carcinoma in pleomorphic adenoma)

This tumour is characterized by a marked polymorphism and displays a different pattern from area to area In some areas there are solid cords and rows of epithelial cells of the squamous variety, atypical in size, shape and nuclear characteristics, as well as in the nuclear-cyto plasmic ratio Mitotic figures are numerous In some other areas, the neoplastic cells tend to constitute tubular-like formations and also small cysts Many mitoses are also present The origin of this tumour has been largely debated, though today it is believed to originate from myoepithelial cells, which stain with T A P colouring (tannin acid phosphotungsten) and h P T A H (phosphotungstic acid haema [illegible])

In the larynx too, when a pleomorphic adenoma or mixed tumour undergoes malignant transformation, it may take the aspect of any malignant neoplasm of the salivary gland type A few cases of such neoplasm arising in the larynx have been described (McDonald & Havens, 1948, Sabri & Hajjar, 1967, Ferlito, 1970[1], Bomer & Arnold, 1971) The neoplasm proved to be clinically highly malignant, and in the case reported by the present writer the female patient died for rupture of the carotid artery about one year following laryngo pharyngo-oesophagectomy McDonald and Havens (1948) in an ample series of 339 cases of salivary gland cancers of the head and neck diagnosed at the Mayo Clinic in 1948, reported two malignant mixed tumours of the larynx

[1] This case has not been included in the present series as it had been evidenced by the author at the Verona Section of the ORL Department of Padua University

12. Mucoepidermoid carcinoma
(or mixed epidermoid and mucus-secreting carcinoma)

It is characterized by clumps and strands of squamous cells, mucus-secreting cells and poorly differentiated cells, the latter being also called cells of intermediate type or transitional cells The above three basic types of cells are present in a varying proportion from area to area in the same tumour, according to the degree of cellular differentiation Many cells contain a variable amount of glycogen The stroma consists of fibrous connective tissue This tumour often shows a cystic or microcystic pattern, and the cystic cavities are lined with mucus secreting cells Their secretion, that is, mucin, reacts positively with mucicarmine and with the PAS reagent after diastase digestion The squamous cell component of the tumour may show, though rarely, intercellular bridges and occasional kera tinization, whereas epithelial pearls are an exceptional finding At times the neoplasm may display follicular structures filled with eosinophilic and mucicarminophilic material thus resembling a tumour of the thyroid gland This is the *follicular variant* of mucoepidermoid carcinoma The present writer has recently evidenced two neoplasms of this type (Ferlito, 1975*c*) In other cases, the tumour is characterized by numerous clear and hydropic cells and this aspect may resemble hypernephroma Clear cells may also be present in some squamous carcinoma The clarity of the cytoplasm of these cells (PAS negative) is probably due to hydropic degeneration This tumour may originate from a pre existing mixed tumour It is rarely seen in the larynx and is probably of ductal origin

This uncommon neoplasm has been described by Arcidiacono & Lomeo, 1963, Cady et al, 1968, Muratti, 1969, Frable & Elzay, 1970 Thomas, 1971, Spiro et al, 1973, Whicker et al, 1974, Davis & Beck, 1975, Sessions et al, 1975, Spiro et al, 1976

The limited number of cases so far reported does not allow conclusions to be drawn on the

biology and prognosis of this rare histologic type of tumour which, however, seems to have a better prognosis than that of the commoner squamous cell carcinoma At present, there are differences in malignancy of mucoepidermoid carcinomas depending on the degree of histologic anaplasia of the neoplasm Radiation therapy is not recommended The accepted treatment is surgical

13. Adenoid cystic carcinoma (or cylindroma)

The tumour is composed of small, basophilic uniform cells with large ovoid nuclei and scanty cytoplasm, arranged in cords or islands in which microcystic spaces resembling Swiss cheese may be seen Occasionally, however, the tumour is made up by solid masses The cystic areas may contain strongly eosinophilic, PAS-positive, mucicarmino-positive and/or hyaline matter This tumour is identifiable essentially for its stroma which acquires a myxoid, or mucinous, or hyaline aspect This tumour has the tendency to invade perineural lymphatics It originates from sero-mucous glands present in the laryngeal mucosa Therefore, in its initial stage this tumour may appear as a submucosal nodule with an intact mucosal lining

Cases of adenoid cystic carcinoma of the larynx have been evidenced by Broeckaert, 1913, Bourgeois & Soulas, 1931, Eigler, 1932, Beck & Guttman, 1936, Lemaître et al, 1936, Kramer & Som, 1939, Havens & Parkhill, 1941, Ide & Cahn, 1948, McDonald & Havens, 1948, Owens, 1949, Pirodda, 1951, Berdal & Mylius, 1954, Putney & McStravog, 1954, Abercromby & Rewell, 1955, Ahued, 1956, Pietrantoni & Leonardelli, 1957, Soboroff, 1959, Suehs, 1960, Leroux-Robert et al, 1961, Ash et al, 1964, Iosipescu et al, 1965 Leroux-Robert & Courtial, 1965 Bardwil et al, 1966, Rosenfeld et al, 1966 Toomey, 1967, Cady et al, 1968, Dallachy, 1969, Berdal et al, 1970, Leonardelli & Pizzetti, 1970, Adams & Duvall, 1971, Pincini & Mandelli 1971 Olofsson & van Nostrand, 1973, Spiro et al, 1973, Muzaffar & Bolstad, 1974, Whicker et al, 1974, Fechner, 1975, Gerard & De Gandt, 1975, Sessions et al, 1975, Houle et al, 1976, Spiro et al, 1976

Table V. *Clinical comparisons in 27 cases of cylindroma and other adenocarcinomas*

	Cylindroma (9 lesions)	Other adenocarcinomas (18 lesions)
Age	47 years	61 years
Males	6 (67 %)	16 (88 %)
Duration of symptoms	29 months	15 months
Subglottic origin	8 (90 %)	2 (10 %)
Initial enlarged cervical nodes	1 (10 %)	6 (66 %)
Average survival	103 months	52 months

From Whicker et al, 1974

This tumour is the most common malignant neoplasm of the laryngeal glands (Friedmann & Osborn, 1976) Leonardelli & Pizzetti (1970) wrote that of their four cases, only one patient had died three years later for cachexia following metastatic spread, whereas the other three patients were alive 3, 4 and 5 years respectively after diagnosis was made

Leroux-Robert et al (1961) found 9 cases of adenoid cystic carcinoma from a series of 1 200 operations

These tumours are reported to have a poor tendency to metastasize to lymph nodes, but are known to spread to the lungs, as in the cases recently reported by Gerard & De Gandt (1975)

According to Whicker et al (1974) the average survival of adenoid cystic carcinoma or cylindroma is better than that of other adenocarcinomas (indeterminate type, mucoepidermoid carcinoma)

The elective treatment is surgery, whereas radiation therapy must be employed only as a complementary treatment

In Table V some significant data on adenocarcinomas are reported

14. Acin cell carcinoma (or acin cell adenocarcinoma)

It is characterized by the presence of round or polygonal tumour cells arranged in sheets or acinar groups The cytoplasm is abundant,

granular, more or less markedly basophilic, resembling the cells of normal serous acini Clear cells and vacuolated cells may also be present, but this is a finding which occasionally characterizes the whole tumour The typical cells are of the acinic variety—they are negative to Alcian blue and to mucicarmine, but are almost always PAS-positive, though the intensity of the reaction may vary from area to area in the same tumour and from tumour to tumour These neoplastic cells do not contain glycogen, apart from minute amounts demonstrable by electron microscopy, in fact their cytoplasm remains positive to PAS stain even after diastase pre-treatment The nucleus is small, hyperchromatic and does not have a constant position, though it is often located peripherally Tumour stroma is generally very scanty, but sometimes may be more abundant and may show occasional hyalinisation At times, lymphoid tissue with follicles and germinal centers may be noted in a vascular tumour stroma Laminated calcified structures resembling psammoma bodies may be present both in the stroma nd in the epithelium Undifferentiated areas d necrotic foci are only rarely observed Mitoses are rare and the neoplasm, which may metastasize to regional and distant lymph nodes, is often delimited by a fibrous connective tissue capsule In the same tumour, trabecular, microcystic, papillar, solid and adenocarcinomatous aspects may be simultaneously present Only rarely does the neoplasm show spaces containing an eosinophilic, PAS-positive matter which resembles colloid Such pattern has a similarity to thyroid t ssue Differential diagnosis—not easy at all—must be made from mucoepidermoid carcinoma (the neoplastic cells of this tumour seem to contain abundant glycogen), from clear epidermoid carcinoma and from laryngeal metastases of clear cell carcinoma of the kidney The tumour may al o be mistaken for a meta stasis from a thyroid carcinoma when follicular structures are present

The neoplasm originates either from serous cells of salivary glands acini or from the intercalated duct cells It is an extremely rare tumour and personally I have evidenced only one case arising in the larynx Recently, Montes Noriega (1974) has described one case of such lesion located in the aryepiglottic fold

15. Carcinosarcoma

This is an extremely rare tumour, though several cases have been described in the literature In our opinion, however, these cases are anaplastic carcinomas, with pseudocarcinomatous reaction of the stroma (see 2 2)

The tumour consists of an admixture of carcinomatous and sarcomatous elements Certainty of diagnosis may be attained when epithelial and connective malignant elements are found in metastases The neoplasm is extremely rare in the larynx, and in my opinion most of the cases described must be considered as anaplastic carcinomas with a pseudosarcomatous reaction of the stroma Recently, Minckler et al (1970) reported a case of "true" laryngeal carcinosarcoma which had metastasized as a carcinoma and sarcoma Ash and Raum (1956) illustrate a case of fibrosarcoma adjacent to invasive carcinoma Batsakis (1974) reports a case of rhabdomyosarcoma and squamous cell carcinoma in the same patient I have seen one example of associated squamous cell carcinoma in situ and malignant pleomorphic fibrous histiocytoma of the larynx (Ferlito, 1976d) These last three cases must be considered as "collision tumours", that is, they are two distinct neoplasms only accidentally present in the same organ, but having a different histogenesis It should be borne in mind that the term "carcinosarcoma" should not be confused with that of "collision tumour" which means that two different tumours arisen separately have later become admixed

Carcinosarcoma is a mixed neoplasm (mesenchymal and epithelial) and in order to establish such a diagnosis correctly, it is necessary that not only "true" elements of carcinoma and sarcoma be simultaneously present in the tumour, but also that they originate from a single stem cell with divergent differentiation

The latter aspect however must not be confused with the false sarcomatous aspects that can be seen sometimes in some malignant epithelial neoplasms

16 Unclassified carcinoma

This definition should group all primary larynx cancers that cannot be placed in any of the type categories described above When the pathologist is unable to label a tumour correctly it is preferable to define the neoplasm as unclassified tumour instead of defining it wrongly This may avoid inadequate treatment It should also be advisable to submit laryngeal unclassified carcinomas to experienced pathologists particularly trained in ENT pathology

IV. Discussion and Conclusions

The histopathological study that we have conducted is the first step of a sort of re-consideration of the whole question of laryngeal and hypopharyngeal tumour disease now under way at our department It is already possible, however, to give indications and draw conclusions which we believe are useful for establishing a more appropriate treatment of the disease Though schematisms on this subject may be considered either too pretentious or simplistic, we think it advisable to lay stress on the most important points from the anatomophatological and clinical viewpoints

a. Limits of tumour classifications

First of all it must be said that all classifications of a multi-faceted disease such as the malignant neoplasm are always liable to criticism Classifications are also easily modifiable, though the pathomorphological criteria adopted are almost identical On the other hand, it appears necessary nowadays to define in the best possible way the histologic type of tumour, so as to help the clinician in his difficult choice of the treatment (surgery—radical or conservative—, radiation or immunotherapy)

A variant of a histologic type of tumour may well cause some difficulties to the clinician, who is used to establish treatment for the commonest types of cancer and on the basis of the site and extent of the malignant disease This is why the Clinician Pathologist Team is becoming more and more actual as experience has proven at our Department, and their mutual co operation is increasingly felt necessary both for establishing diagnoses and treatments, and not only in the cancer field

The correct identification of some histologic types of tumour, such as the oat cell carcinoma, allows a better assessment of the malignancy displayed by these neoplasms as compared with the most common squamous cell carcinoma Conversely, the suspected presence of a verrucous squamous cell carcinoma may correctly direct the surgeon in removing a biopsy specimen, as he will not be satisfied by a "negative" histopathological report because he knows that the lesion is actually neoplastic, even though of a little malignancy

The macroscopic aspect (exophytic, ulcero-exophytic, sessile, invasive, ulcero invasive, verrucous) *and the microscopic appearance of the tumour are necessarily connected with the biologic behaviour and clinical characteristics of the lesion, as well as to its prognosis, and constitute, together with other factors, discriminatory elements in the choice of the treatment*

All patients with laryngeal cancer are wrongly considered as having the same disease On the contrary, each tumour has its own distinctive characteristics, therefore it has to be considered as a distinct entity Treatment depends upon a correct diagnosis and must not be always the same It is understood, for instance, that the elective treatment for a laryngeal lymphoma is radiation therapy (Anderson et al, 1976), whereas the same treatment may be harmful or ineffective in case of verrucous squamous cell carcinoma (Kraus & Perez-Mesa, 1966, Biller et al, 1971, van Nostrand & Olofsson, 1972, Ogura & Biller, 1973, Bauer, 1974, Ferlito, 1975*b*, Hyams, 1975*b*, Ferlito et al, 1976) An undifferentiated carcinoma needs, in addition to ample excision of the laryngeal lesion, radical neck dissection (in this case the larynx and the cervical lymph nodes constitute an inseparable neoplastic entity), whereas removal of lymph nodes is not required for a patient with verrucous squamous cell carcinoma

Those patients with malignant laryngeal neoplasm, who are candidates for supraglottic

laryngectomy on the basis of the macroscopic extent of the tumour, must in our opinion undergo total laryngectomy all the same, if a preoperative biopsy has shown an undifferentiated carcinoma Supraglottic laryngectomy must not be performed only on the basis of the macroscopic extent of the tumour, as is currently believed nowadays by most surgeons, but must be adopted only if there are favourable biopathological factors for its choice It is wrong to define with millimetrical preciseness the limits of the surgical techniques without evaluating the biological malignancy of the neoplasm and the tumour host relationship (Sala, 1976*c*) The present conservation surgery of the larynx is based on wrong considerations The proliferation of conservative surgical techniques must not be encouraged till the bio pathological problems connected with the malignant disease are better known (Ferlito, 1976*f*) This is by no means an underestimation of the conservation surgery of the larynx This surgery needs only a biological rationalization for its adoption, so as to select the candidates and to avoid the so called recurrences which in most cases, and particularly in those occurring within a year, are but residual carcinomas

We cannot agree with those authors adopting conservation surgery also in cases for which the histological examination of the surgical specimen shows clearly that excision of the tumour has *not been as radical as it* should have been, though in a few rare cases, for immunological factors, the body is capable of fighting the residual cancer cells in the portion of the larynx that has not been removed If an operation of "tumorectomy" is liable to criticism, it is even more unacceptable, both biologically and from a human point of view, to perform an "incomplete tumorectomy", perhaps with complementary radiation therapy

The most advanced surgery must not forget that the ultimate purpose is to save the life of the patient, because if this was not so, any therapeutic, social or human initiative would be meaningless If, on the contrary, there is the possibility to perform a conservation treatment which is also biologically rational and with the same results that may be obtained with total laryngectomy, then it is not only justified but mandatory to adopt it

It appears clearly, then, that adequate treatment cannot be established unless the histologic type of tumour has been correctly identified—a basic condition for establishing any treatment *There is not "the" cancer of the larynx, there are cancers of the larynx, each of them displaying a different degree of biologic activity* Each histologic type of cancer has specific prognostic implications and therefore the therapeutic modality selected should be tailored to the nature of the neoplasm

It is worth mentioning that the larynx may be the site of origin of malignant epithelial neoplasms found at times in the lung (oat cell carcinoma, carcinoid, giant cell carcinoma, clear cell carcinoma, beside anaplastic carcinoma, squamous cell carcinoma, mucoepidermoid carcinoma, adenoid cystic carcinoma, carcinosarcoma, etc), though the percentage of distribution is different This fact must not strike if we consider that both organs arise from the medial diverticulum of the foregut

Furthermore, the larynx may exhibit malignant neoplasms typical of the salivary glands, such as the malignant mixed carcinoma, mucoepidermoid carcinoma, adenoid cystic carcinoma, acin cell carcinoma and adenosquamous carcinoma This is due to the fact that usually mucous glands of the exocrine compound tubulo alveolar type, the topography of which has been carefully studied by Malkevich (1967) and by Nassar & Bridger (1971), are present in the laryngeal mucosa Johns et al (1973) have also described a tumour of the larynx consisting of large cells with abundant eosinophilic cytoplasm that were histochemically (PTAH) negative for mitochondria For this reason the authors use the term "oncocytoid carcinoma" The neoplasm was not a true oncocytic carcinoma but carcinoma of mucous gland origin, because the cells had no minimal histochemical requirements of the oncocyte

b. Need for a comprehensive evaluation of the tumour (not only on the basis of the TNM system)

Not only should the pathologist correctly identify the histologic type of tumour, but also provide the clinician with a more detailed pathomorphological evaluation This is possible by means of the newest techniques in histopathology tending to outline the tumour lesion in all its aspects, thus allowing a better assessment of the prognosis and the choice of the most adequate treatment In each biopsy report, the following elements must be indicated histologic type, histologic grading of malignancy and cellular response In some cases, these three factors are consequential, as seen for example in case of verrucous squamous cell carcinoma which is, as said before, a histologically particular neoplastic lesion, with a high degree of cellular differentiation, a low grade of histologic malignancy and an intense cellular immune response in tumour stroma, or in what may be said to be the opposite condition—in case of undifferentiated tumours characterized by poorly differentiated cells, high grade of histologic malignancy and an almost absent lymphoplasmacellular immune response Lymphocytic infiltration is the morphological equivalent of the host s cell mediated immune response against the neoplasm entailing a more favourable prognosis and therefore a longer survival Conversely, the absence of lymphocytes in tumour stroma indicates the opposite prognostic outcome Lymphoplasmacellular infiltration must be regarded as a valuable expression of the body's reactive potential against tumour Table VIII shows the structural biological characteristics of the two lesions representing the two most typical, opposite conditions

In most laryngeal cancers, however, the combination of the three factors varies, their proportion may also vary in the course of the disease, if long lasting These variations are caused by factors altering the mechanism of cellular immunity, such as acute virus diseases, chronic infectious diseases (tuberculosis, syphilis), radiation therapy, chemotherapy, etc The problem is not restricted to the primary tumour, but involves also the behaviour of regional lymph nodes At our department, a systematic investigation is being conducted on the behaviour of cervical lymph nodes removed at operation together with the tumour The morphologic study of lymph nodes draining the tumour allows the immunologic assessment of cancer patients Useful prognostic indicators may be discovered from this study Histologically, lymph nodes exhibit a varying type of reaction corresponding to a varying degree of immune response

A lymph node reaction characterized by a pattern of "lymphocyte predominance" witnesses an immune response of the cell mediated type, histologically expressed by a marked hyperplasia of T-lymphocytes located in the deep cortical regions The prognosis for patients with this lymph node pattern is better than that of patients in whom a humoral immune response predominates and may be morphologically assessed when a pattern of "germinal center predominance" is seen, that is, an immune response in the form of expanded thymus independent outer cortex areas containing B-lymphocytes The cortex and the medulla of the lymph node are associated with humoral immunity, whereas the paracortex is responsible for cell mediated immune response and therefore is considered a thymus-dependent area The presence of epithelioid cells is an expression of cell mediated reaction (Ferlito, 1976*c*)

As to the pattern of sinus histiocytosis, it does not appear to have a relation with host survival and evolution of the tumour disease (McGavran & Bauer, 1975)

Bennet et al (1971) and Futrell et al (1971), however, did not find in their patients with cancer of the larynx and hypopharynx a clear correlation between prognosis and the morphologic changes in regional lymph nodes

Our investigations are confirmed by a recent paper by Berlinger et al (1976*a*) who studied a group of 84 patients with squamous cell carcinoma of head and neck regions, forty two

having a cancer of the larynx Sections of regional lymph nodes draining the neoplasms were examined microscopically to assess the morphologic pattern of response The prognosis was definitely better for patients displaying lymphocyte predominance, whereas none of the patients whose lymph nodes showed the depleted pattern survived 5 years (see Table VI)

Berlinger et al (1976*a*) stress that the percentage of patients with histologically proven lymph nodes metastases showed a correlation with their lymph node patterns, in that the patients with lymphocyte depletion had metastases in 100 % of cases, whereas this percentage dropped to 23 % in patients whose lymph nodes displayed a pattern of lymphocyte predominance

These data mean that a relationship exists between immunologic activity, progression of the neoplasm and survival of the host

It appears to be helpful to assess the immunologic defensive potential of the cancer patient because the therapeutic programme must not, in order to be biologically rational, disregard the host's immunologic defensive potential At present, it is not easy to typify immunologically a cancer patient, in spite of the ever growing number of tests tending to assess his immunologic configuration Actually, these tests are non specific The most common immunologic tests to evaluate the host's immune response are BCG (Bacillus Calmette Guerin), PPD (purified protein derivative, an extract of M tuberculosis), DNBC (dinitrochlorobenzene), SKSD (streptokinase-streptodornase), Candidin, Mumps lymphocyte blastogenic transformation to phytohemagglutination (PHA), T-cell rosette test etc but there is no single test of overall cellular immune-function (Eastham et al, 1976) Some researchers (Conticello et al, 1972, Serrou et al, 1972 Catalona & Chretien, 1973, Mandel & Kiehm, 1974 Twomey et al, 1974) have observed that there is a high incidence of anergy to DNBC in patients with head and neck cancers Harris (1976) states that though a negative reaction with skin testing is seen in anergic patients, the results of these tests often disagree with

Table VI *Five-year survival as a function of lymph node pattern in squamous cell carcinoma of the larynx*[a]

Lymph node pattern	No of patients	Alive	% survival
Lymphocyte predominance	20	18	90
Germinal center predominance	7	6	86
Unstimulated	13	7	54
Lymphocyte depletion	2	0	0
Total	42	31	74

[a] Statistically significant by chi square, $p < 0.01$
From Berlinger et al, 1976*a*

results of cell-mediated immunity tested in vitro procedures

The ECOG (Eastern Co-operative Oncology Group—a multi-institution cancer study organization with participating institutions in North America, Europe and Africa) uses the four test preparations for the initial immunological assessment of cancer patients

1) PPD,
2) Candida albicans extract,
3) Mumps antigen,
4) Varidase (streptokinase-streptodornase)

It must be emphasized that none of the immunologic tests currently adopted allows the immunological assessment of a cancer patient, since the tests are non specific (Niksik et al, 1976) and their significance is often difficult to be established We are of the opinion that more than a single immunologic test, the evaluation of the defensive activity of immunocompetent cells in tumoral and peritumoral stroma, which can be assessed also in biopsy specimens (Sala & Ferlito, 1976) and the reaction of regional lymph nodes (Zechner, 1975, Berlinger et al, 1976*a*, *b*, Ferlito, 1976*c*) should be considered as an expression of host immune response The favourable prognosis attached to the presence of cellular response in cancer of the larynx is also supported by various authors (Cali' et al, 1968, 1972, Bennett et al, 1971, Koselnik Glugla &

Krygier-Stojalowska, 1975, Zechner, 1975, Sala & Ferlito, 1976)

Paavolainen (1970) does not find in laryngeal cancer that a correlation exists between plasma cell and lymphocyte infiltration and the prognosis, whereas the same author (Paavolainen et al, 1973) state that such correlation exists in epidermoid carcinoma of the tongue

Also the Committee for Radiation Oncology Studies (1976) considers the degree of infiltration by lymphoid elements in histologic biopsy specimens to be an anticancer "specific" immunoreactivity test "in vivo", whereas other tests in vivo (complete blood counts, T+B cells, Recall antigens, DNBC, γ globulin, complement) and in vitro (polymorphonuclear leukocyte and monocyte function and lymphocyte transformation) are "general' immunoreactivity tests

The results of a histopathological evaluation of local lymphoplasmacellular immune response and the immune reaction in lymph nodes seem to us to be reliable indicators of the host's immune potential We believe that developments in immunobiology in the near future will make it possible to better investigate the immune potentiality of a cancer patient and to assess it more correctly than possible at present Practically, it ought to be possible to typify a cancer patient immunologically

The fact that a cancer lesion is clinically and biologically unpredictable in its behaviour is often due to its being studied only superficially The malignancy of a neoplasm is strictly connected to various factors concerning partly the lesion itself (intrinsic factors) and partly its host (extrinsic factors) The intrinsic factors are often correlated one another and the most important, besides the site and extent of the lesion, are the histologic type and the histologic grading of malignancy

Histologic type

A correct identification of the oncotype must not be intended as a knowledge of the structure of the neoplasm, but must result in a degree of malignancy, which is different from case to case It is a well known fact that an undifferentiated carcinoma metastasizes more frequently than a differentiated squamous cell carcinoma, and that a verrucous squamous cell carcinoma is less malignant than the common squamous cell carcinoma Each of the above three neoplasms have their own degree of intrinsic malignancy strictly connected to the structure of the neoplasm, regardless of the host's immune potential

Histologic grading of malignancy

This is another important parameter evaluated by taking into consideration the cyto-histologic structure of the tumour Factors allowing a better assessment of the histologic grading of malignancy are, in our opinion, the following

a) *degree of structural differentiation*—As is known, the more the cytologic characteristics of tumour cells resemble normal cells, the more the tumour is differentiated, and vice-versa Thereby the more differentiated the tumour, the lower its intrinsic biologic malignancy This appears also from McGavran et al (1961) and from tables II, III and IV Sessions (1976) says that for glottic and inferior hypopharynx cancers there is a significant correlation between tumour differentiation and positive lymph nodes metastases

b) *frequency of mitoses*—Mitoses are the expression of cell multiplication and thereby of tumour growth The mitotic activity is better assessed peripherally in the tumour mass, where the invasion of the neighbouring healthy tissue may be adequately evaluated A high mitotic index is an expression of high biological malignancy

c) *cellular and nuclear pleomorphism*—This feature shows the degree of homogeneity of neoplastic cells and of their nuclei The biologic malignancy of a tumour increases with the increasing pleomorphism of its cell population The nuclear grading is a specific prognostic factor and may be evaluated as NG1 (the least favourable), NG2 (intermediate between NG1 and NG3) and NG3 (the most favourable)

d) *necrosis*—In tumours not treated by X-rays, the presence of areas of necrosis is a morphologic indicator of malignancy since it means that the tumour grows quickly and has not a sufficient blood supply Necrotic foci are most often seen in undifferentiated carcinomas and oat cell carcinomas They are less frequent in well differentiated tumours,

e) *invasion*—An incision biopsy allows to assess the tumour invasion, which differs from case to case As an example, in carcinoma "in situ" the tumour invasion does not go beyond the basement membrane and in the verrucous squamous cell carcinoma, invasion is limited, since the margins of the tumour are of a pushing rather than an infiltrating type The mode of invasion (clear-cut margins or shaded margins, diffuse lesion) is not to be disregarded in establishing the malignancy of a neoplasm

According to Jacobsson et al (1973) the histologic grading of malignancy in carcinoma of the larynx may be assessed in the initial biopsies, particularly if the laryngologist performs incision biopsies, as currently done at our Department

The above authors say that at the Radium hemmet and at the Department of Tumour Pathology of the Karolinska Sjukhuset in Stockholm, eight morphologic criteria are taken into consideration for establishing the histologic grading of malignancy of a laryngeal tumour Four of them represent the neoplastic cell population itself (structure, differentiation, nuclear polymorphism and mitoses) whereas the remaining four criteria represent the tumour-host relationship (mode of invasion, stage of invasion, vascular invasion, cellular response) These parameters served as a basis for evaluation of a series of 230 patients with glottic carcinoma of the larynx treated by radiotherapy and followed-up for at least 5 years The prediction of the 5 year results recurrence or not, was better than that obtained with the TNM system (Jakobsson, 1975)

As a support to what has just been said, it must be emphasized that never have we evidenced distant and cervical lymph nodes metastases from a case of verrucous squamous cell carcinoma, and this tumour, which is a variant of squamous cell carcinoma, is characterized by a high degree of cellular differentiation This fact is also confirmed by other investigators (Biller et al, 1971, van Nostrand & Olofsson, 1972, Biller & Bergman, 1975, Hyams, 1975, Weiland, 1975) who have followed up patients with verrucous squamous cell carcinoma Fisher (1975) only has evidenced 5 cases out of 31 of verrucous squamous cell carcinoma of the larynx, with lymph nodes metastases

For this purpose, some reservations must be made on the cases of verrucous squamous cell carcinoma reported by Fisher (1975), not only because this tumour accounts for 11% of a group of 276 infiltrating carcinomas of the larynx (this is the highest percentage so far reported in the world literature) but also because squamous cell carcinomas grade I found in this series represent only 5% The relatively high incidence of lymph nodes metastases (5 cases) observed by Fisher (1975) might well be due, in my opinion, to the fact that the author has classified as verrucous squamous cell carcinoma also some well differentiated squamous cell carcinomas the course of which is less favourable The author himself explains that it is not always easy to distinguish the aspects typical of the verrucous squamous cell carcinoma from those of the well differentiated squamous cell carcinoma, and that in six cases of his series there was a small region interpretable as grade I squamous cancer So far, no case of verrucous squamous cell carcinoma of the larynx has been reported to have given lymph nodes metastases From the series by Kraus & Perez Mesa (1966), of 105 verrucous squamous cell carcinoma of the oral cavity (77), larynx (12), glans penis (8), nasal fossae (4), vulva (1), vagina (1), scrotum (1), perineum (1), none had shown lymph nodes metastases, apart from 4 patients treated by radiation, apparently as a result of alteration of the biologic character of the neoplasm Hyams (1975) reports that no lymph node metastases were evidenced in a

series of 29 patients with verrucous squamous cell carcinoma of the larynx

Also in tumours of connective tissue origin, such as the laryngeal fibrosarcoma, there is a clear correlation between the degree of cellular differentiation and survival The prognosis is very good with ample excisional therapy in well differentiated fibrosarcoma, whereas in the poorly differentiated tumours the prognosis is unfavourable and all patients in the cases reported in the literature died within three years of therapy (Flanagan et al, 1965)

Investigations in the field of kinetics of cell growth have demonstrated that a sort of dichotomy exists between cell proliferation and cellular differentiation, in that an undifferentiated tumour grows more quickly than a well-differentiated neoplasm Therefore, the growth potentiality of a neoplastic lesion is indirectly proportional to its grade of differentiation As a result, the most differentiated tumours have a doubling time longer when compared with undifferentiated carcinomas The growth of a tumour lesion is connected to the structural differentiation of the neoplasm

As to the evaluation of cellular differentiation of laryngeal carcinomas, we agree with Mc Gavran et al (1961) who think that the character of the laryngeal carcinoma with regard to differentiation can be adequately evaluated by biopsy In fact, our experience induces us to say that only exceptionally has the study of the whole tumour, as seen in sectioned laryngectomy specimens, shown a varied pattern of differentiation

In addition to these tumour intrinsic factors, there are factors connected with the immune response of the host on which the prognosis and survival of a cancer patient depend These factors are the degree of the host's immune response which is tested by taking into consideration the defensive activity of immunocompetent cells present in tumoral and peritumoral stroma (Cali et al, 1968, 1972, Bennett et al, 1971, Koselnik Glugla & Krygier-Stojalowska, 1975 Ferlito, 1976*f*, Sala & Ferlito, 1976) and the morphology of lymph nodes draining the neoplasm, in relation to immunological function—cellular and humoral immunity (Berlinger & Good, 1974, Cortesina et al, 1974, Berlinger et al, 1976*b*, Ferlito, 1976*c*)

Summing up, the biologic behaviour of the larynx cancer and therefore the prognosis and the survival of the patient depend not only on the site and extent of the neoplasm, but also on other basic biopathological factors, that is, the histologic type, the histologic grading of malignancy and the immune response of the host Therefore, treatment of a malignant neoplasm of the larynx or hypopharynx must be based on the clinical staging as well as on the histologic grading The latter factors may be of a basic importance for some oncotypes, so that the clinician is led to choose a certain type of treatment directly from the histologic diagnosis, irrespective of the staging of the lesion It appears therefore that factors conditioning the course of a laryngo-hypopharyngeal cancer and allowing the establishment of the prognosis (cancer is no longer to be considered as an unpredictable disease) are the following

1) *Staging of the disease*, which indicates tl site and extent the tumour and may l assessed on the basis of the TNM systen This classification defines the extent of tl disease in terms of three components a) th primary tumour, designated by letter T an expressed in terms of the extent of spread c the cancer from the primary site and mobilit of the larynx, b) the regional lymph node designated by letter N, and c) distant meta stases designated by letter M,
2) *Oncotype*,
3) *Histologic grading*,
4) *Immuno-morphologic findings*, evaluated by considering the cellular immune response, that is, the presence of immunocompetent cells in tumoral and peritumoral stroma graded as marked, moderate or minimal and the behaviour of lymph nodes draining the tumour Hyperplasia of the thymus-dependent deep cortices (cellular immunity)

Table VII *Anatomo-clinical, biopathologic and immunologic factors conditioning the prognosis of laryngo-hypopharyngeal cancer*

Factor			
SITE	TUMOUR INTRINSIC FACTORS	Clinical staging	Mono or multifocal
EXTENT			This means not only the size of the primary neoplasm or neoplasms, but also their nodal and visceral metastases
ONCOTYPE			
HISTOLOGIC GRADING OF MALIGNANCY		Histologic grading	Degree of structural differentiation Frequency of mitoses Cellular and nuclear pleomorphism Necrosis Invasion
IMMUNE RESPONSE OF HOST	HOST FACTORS		Cellular response (plasmolymphocytic) in tumoral and peritumoral stroma Morphology of lymph nodes draining the neoplasm in relation to immunological function (lymphocyte predominance cellular immunity, germinal center predominance – humoral immunity, unstimulated, lymphocyte depletion)

entails a better prognosis and a consequent longer survival than observed when hyperplasia of the germinal centres of the thymus-independent cortical area and the medulla (humoral immunity) is seen

Table VII is a synoptic view of what has been described

The systematic histopathological study of the surgical specimen (primary lesion and cervical lymph nodes) greatly helps in understanding the biologic behaviour of the tumour Now, this is a current practice at our department, with a view to establishing a correlation between histology on one side and the results of treatment and long term prognosis on the other

c. Need for co-operation between the pathologist and the clinician

Data reported in the present study demonstrate that an up to date approach to the neoplastic disease is only possible by means of a constant co operation between the pathologist and the clinician The pathologist must examine problems also from the clinical point of view and the clinician must in turn take a biopathological approach to the disease in the broadest sense of this term (biologic, histopathologic, immunologic approach) The treatment of a neoplasm is never a problem of surgical technique only, but also a biologic problem

A close co-operation between the pathologist and the clinician, who must examine the lesion in all its aspects, makes the course of the disease less unpredictable As a rule, there is a correlation between gross morphology of the tumour and its histopathological pattern evaluated in its entirety Usually, exophytic tumours in which outgrowths prevail over ulcerated features, are malignant tumours with a marked cellular response, whereas deeply ulcerated tumours, though small in size, with macroscopic necrotic foci and intense oedema of the surrounding tissue, are almost always the result of a deficiency in immune response, and the pathologist will be able to find a histological evidence of such defective immune reaction by considering not only the histologic type, but also the histologic grading of cellular differentiation and the

series of 29 patients with verrucous squamous cell carcinoma of the larynx

Also in tumours of connective tissue origin, such as the laryngeal fibrosarcoma, there is a clear correlation between the degree of cellular differentiation and survival The prognosis is very good with ample excisional therapy in well differentiated fibrosarcoma, whereas in the poorly differentiated tumours the prognosis is unfavourable and all patients in the cases reported in the literature died within three years of therapy (Flanagan et al, 1965)

Investigations in the field of kinetics of cell growth have demonstrated that a sort of dichotomy exists between cell proliferation and cellular differentiation, in that an undifferentiated tumour grows more quickly than a well differentiated neoplasm Therefore, the growth potentiality of a neoplastic lesion is indirectly proportional to its grade of differentiation As a result, the most differentiated tumours have a doubling time longer when compared with undifferentiated carcinomas The growth of a tumour lesion is connected to the structural differentiation of the neoplasm

As to the evaluation of cellular differentiation of laryngeal carcinomas, we agree with Mc Gavran et al (1961) who think that the character of the laryngeal carcinoma with regard to differentiation can be adequately evaluated by biopsy In fact, our experience induces us to say that only exceptionally has the study of the whole tumour, as seen in sectioned laryngectomy specimens, shown a varied pattern of differentiation

In addition to these tumour intrinsic factors, there are factors connected with the immune response of the host on which the prognosis and survival of a cancer patient depend These factors are the degree of the host's immune response which is tested by taking into consideration the defensive activity of immunocompetent cells present in tumoral and peritumoral stroma (Cali' et al, 1968, 1972, Bennett et al, 1971, Koselnik-Glugla & Krygier-Stojalowska, 1975 Ferlito, 1976*f*, Sala & Ferlito, 1976) and the morphology of lymph nodes draining the neoplasm, in relation to immunological function—cellular and humoral immunity (Berlinger & Good, 1974, Cortesina et al, 1974, Berlinger et al, 1976*b*, Ferlito, 1976*c*)

Summing up, the biologic behaviour of the larynx cancer and therefore the prognosis and the survival of the patient depend not only on the site and extent of the neoplasm, but also on other basic biopathological factors, that is, the histologic type, the histologic grading of malignancy and the immune response of the host Therefore, treatment of a malignant neoplasm of the larynx or hypopharynx must be based on the clinical staging as well as on the histologic grading The latter factors may be of a basic importance for some oncotypes, so that the clinician is led to choose a certain type of treatment directly from the histologic diagnosis, irrespective of the staging of the lesion It appears therefore that factors conditioning the course of a laryngo-hypopharyngeal cancer and allowing the establishment of the prognosis (cancer is no longer to be considered as an unpredictable disease) are the following

1) *Staging of the disease*, which indicates the site and extent the tumour and may be assessed on the basis of the TNM system This classification defines the extent of the disease in terms of three components a) the primary tumour, designated by letter T and expressed in terms of the extent of spread of the cancer from the primary site and mobility of the larynx, b) the regional lymph nodes designated by letter N, and c) distant metastases designated by letter M,
2) *Oncotype*,
3) *Histologic grading*
4) *Immuno-morphologic findings*, evaluated by considering the cellular immune response, that is, the presence of immunocompetent cells in tumoral and peritumoral stroma graded as marked, moderate or minimal and the behaviour of lymph nodes draining the tumour Hyperplasia of the thymus-dependent deep cortices (cellular immunity)

seemed to us worthy of publication, though they are still being scrutinized and susceptible of improvements By this work of ours, though with its limitations, we have made an attempt to help the pathologists who may not have at their disposal ample series such as those available at our Department

Omissions and errors may be expected in the present classification, due to the vastness and newness of the subject Suggestions are of course welcome We propose such a classification to the attention of interested groups for evaluation and comparison with their own experience, and for the working out of results of prognostic value

V. Summary

This paper is a first attempt to classify the variety of histological types of malignant epithelial tumours occurring in apparently uniform organs such as the larynx and hypopharynx. The commonest and rarest malignant epithelial tumours arising in these organs are described, clinically, histopathologically and prognostically.

Till not long ago, the prognosis of tumours was based, according to the TNM system, only on the site and extent of the tumour (cancers arising in the hypopharynx were considered to have a poorer prognosis). Prognosis and treatment, on the contrary, must be established by taking into account three additional factors, that is, the histologic type, the histologic grading of malignancy (judged upon the degree of structural differentiation, the frequency of mitoses, cellular and nuclear pleomorphism, necrosis, invasion) and tumour-host relationship consisting of lymphocyte and plasma cell infiltration in tumour stroma.

The correct identification of a neoplasm and *the evaluation of the above factors are therefore* extremely important not only for purely scientific purposes, but also for the choice of treatment and for establishing the long term prognosis. Tumour-host interaction in particular must be carefully considered, as it represents the immune system response to tumour invasion. Iatrogenic interference with this factor such as radiation therapy, seems to affect negatively the biologic behaviour of the neoplasm, therefore tumours with marked cellular immune response (such as the verrucous *squamous cell carcinoma) should* be treated surgically.

The systematic histopathological study of surgical specimens (primary lesion and regional *lymph nodes) is now* a current practice at the ORL Department of Padua University, with a view to establishing a correlation between gross morphology, histopathology, treatment and outcome. This work implies a close co-operation between the pathologist and the clinician—a team that has become actual at the author's department. Their constant co-operation has made it possible to establish that each malignant tumour is an entity with its own characteristics needing a "tailor-made" treatment.

The abundant tumour documentation of the ORL Department of Padua University (about two hundred cases yearly in recent years) has made it possible to carry out this study, which *is based on 2 052* laryngopharyngeal neoplasms encountered in the last eleven years. Omissions and errors, however, might have been possible, and therefore the study is submitted to researchers working in the same *field for comparison* and suggestions.

VI. Resumé

:tte étude est une premıère tentatıve de classı-
atıon des tumeurs épıthélıales du larynx et de
ypopharynx Les tumeurs les plus commune
ısı que les plus rares y sont décrıtes des poınts de
e clınıque, hıstopathologıque et du pronostıc
Il n'y a pas longtemps encore, le pronostıc
la maladıe cancéreuse était établı sur la base
la classıfıcatıon TNM quı prenaît en con-
lératıon la localısatıon et l'extensıon de la
ıon (les cancers de l'hypopharynx, bıen que
ıtés, étant consıdérés comme ayant un pro-
stıc moıns favorable) Par contre, le pronostıc
le traıtement doıvent être établıs en examınant
ıs facteurs supplementaıres, c'est a-dıre le
ɔe hıstologıque, le degre de malıgnıté (établı
r la base du degré de dıfferentıatıon struc-
:elle, de la fréquence des mıtoses, du poly-
ɔrphısme callulaıre, de la présence de foyers
crotıques et de l'ınvasıon atteınte par la
neur) aınsı que l'ınteractıon tumeur-malade
L'ıdentıfıcatıon exacte de la tumeur et l'évalua-
ın des facteurs cı dessus est ımportante non
ılement du poınt de vue scıentıfıque, maıs
ssı pour le choıx du traıtement et pour l'eta-
ssement du pronostıc a long terme L'ınter-
tıon tumeur-malade, en partıculıer, doıt être
nsıderee comme etant la reponse ımmunıtaıre
l'organısme vıs à-vıs de la maladıe cancereuse,
ıı se traduıt par une ınfıltratıon de lympho-
tes et plasmocytes dans le stroma tumoral
ɔute ınterference ıatrogene avec le méchanısme
la reponse ımmunıtaıre, comme par exemple
la radıothérapıe, semble affecter négatıvement
le comportement bıologıque de la tumeur. Par
conséquent, le traıtement des tumeurs avec une
réponse ımmunıtaıre consıdérable (comme le
carcınome verruqueux) doıt être chırurgıcal

L'etude hıstopathologıque systématıque des
spécımens opératoıres (lésıon prımıtıve et lym-
phatıques regıonaux) est aujourd'huı une pra-
tıque habıtuelle au département ORL de l'Unı-
versıté de Padoue, dans le but d'établır une
correlatıon entre l'aspect macroscopıque, l'aspect
hıstologıque, le traıtement et les résultats à long
terme Ce travaıl ımplıque une collaboratıon
étroıte entre le pathologıste et le clınıcıen—un
"team" quı est déja une réalıté nécessaıre dans
le département où l'auteur prête son oeuvre
Cette collaboratıon constante a permıs d'établır
que chaque lésıon cancereuse est une entıté
partıculıere necessıtant un traıtement "ad hoc".

La documentatıon consıdérable exıstante au
departement ORL de l'Unıversıté de Padoue sur
les cancers du larynx et de l'hypopharynx
(envıron deux-cent cas par an au cours des
dernıeres annees) a permıs d'effectuer cette
etude, quı est basée sur l'examen de 2052 néo-
plasıes du larynx et du pharynx observées au
cours des dernıers onze ans Naturellement
des omıssıons et des erreurs peuvent avoır été
possıbles, c'est pourquoı l'étude est soumıse à
l'attentıon des rechercheurs agıssant dans le
même domaıne pour une comparaıson avec
leurs résultats et pour des suggestıons

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Fig 1 Carcinoma in situ H & E (original magnification, 40)

Fig 2 *Carcinoma in situ* Detail of fig 1 *Note total disorganization of all layers*, cellular atypism and the intact basement membrane H & E (original magnification, 100)

Fig 3 Well-d fferent ated squamous cell carcinoma H & E. (original magn f cat on, ×40)

Fig 4 Well-d fferent ated squamous cell carcinoma. H gher magn f cation shows two well-def ned kerat n pearls H & E (original magn f cat on, 100)

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Fig 9 Verrucous squamous cell carcinoma Panoramic view Note hyperkeratosis (right) H & E (original magnification, × 16)

Fig 10 Verrucous squamous cell carcinoma Tumour cells deeply infiltrate the mucous membrane H & E (original magnification, × 16)

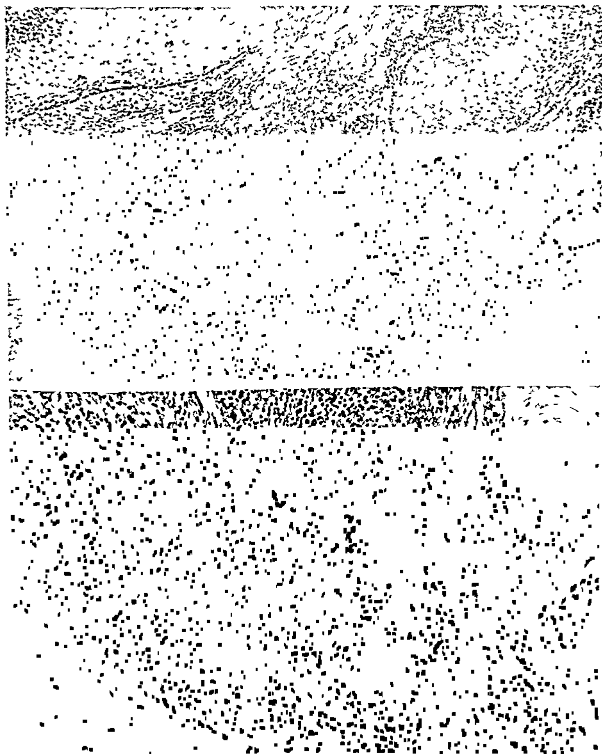


Fig 11 Verrucous squamous cell carcinoma Note abundant inflammatory cells surrounding well-demarcated nests of squamous cell Two lymphatic follicles may be seen H & E (original magnification, ×16)

Fig 12 Verrucous squamous cell carcinoma Connective stroma showing granulomatous tissue including a foreign body giant cell H & E (original magnification, ×40)

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Fig 13. Verrucous squamous cell carcinoma High magnification shows better the relationship between the neoplastic tissue and the marked cellular response. H. & E. (original magnification, ×

Fig 14. Verrucous squamous cell carcinoma Note well-differentiated epithelial
H & E. (original magnification, 100)

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Fig 16 Spindle-cell carcinoma Detail of Fig. 15 Note several bizarre giant cells H & E (original magnification 100)

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Fig 18 Spindle-cell carcinoma Another area of same tumour Higher magnification shows better the anaplastic sarcomatous appearance H & E (original magnification × 100)

Fig 19 Sp ndle-cell carcinoma Note the irregular sp ndle-cell pattern of the tumour and abundant vascularizat on H & E. (original magn f cat on × 40)

Fig 20 Spindle-cell carc noma Deta l of Fig. 19 Note the bizarre anaplast c hyperchromat c g ant cells with several m tot c f gures H & E. (original magn f cation 100)

Fig. 21. Lymphoepithelial carcinoma. Panoramic view. H & E (original magnification, ×16)

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Fg 39 Adenocarcinoma Another tumour area characterized by the presence of glandular struc tures H & E (orig nal magn f cat on, 40)

Fg 40 Adenocarcinoma Deta l of Fg 39 Note cytologic characteristics of tumour cells. H & E (original magnif cat on, 100)

Fig. 49. Clear cell carcinoma. The tumour is characterized by large cells displaying an empty cytoplasm. H & E (original magnification ×40)

Fig. 50. Clear cell carcinoma. Higher magnification shows in some areas abortive glandular differentiation. H & E (original magnification ×100)

Fig 51 Clear cell carcinoma Though the tumour has a solid aspect, its glandular origin may be seen H & E (original magnification × 40)

Fig 52 Clear cell carcinoma Detail of fig. 51 H & E (original magnification 100)

Fig 49 Clear cell carcinoma The tumour is characterized by large cells displaying an empty cytoplasm H & E (orig nal magnificat on 40)

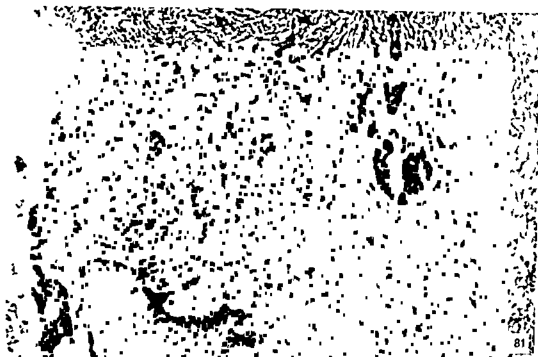
Fig 50 Clear cell carcinoma Higher magnification shows in some areas abortive glandular d f ferentiat on H & E (or ginal magn fication 100)

Fig 51 Clear cell carcinoma Though the tumour has a solid aspect its glandular origin may be seen H & E (original magnification 40)

Fig 52 Clear cell carcinoma Detail of fig 51 H & E (original magnification ×10

Fig 49 Clear cell carcinoma The tumour is characterized by large cells displaying an empty cytoplasm H & E (orig nal magn f cation 40)

Fig 50 Clear cell carcinoma Higher magn f cation shows in some areas abortive glandular d f ferent at on H & E (original magnificat on 100)



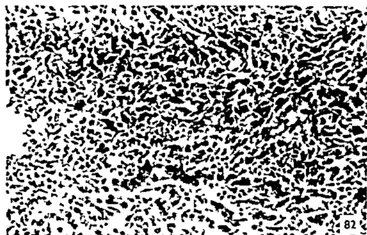


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Fig 82 Carcinosarcoma Liver metastasis Note malignant spindle cells H & E., × 160 (Courtesy of Dr H T Norris, Dept of Pathology of the Univ of Washington School of Medicine, Seattle — From Minckler, D S, Meligro' C H and Norris, H T 'Carcinosarcoma of the Larynx — Case Report with metastases of epidermoid and sarcomatous elements", Cancer, 26, 195–200, 1970)

Photomicroscope III Carl ZEISS (Oberkochen W Germany) with planapochromatic objectives and automatic photographic device 24 × 36 was adopted to obtain the photomicrographs

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Precancerous Lesions of the Larynx

Round Table Symposium
Trieste, Italy, 12 April, 1975

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ANDREA BOSATRA

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Preface

I am very glad to welcome here my former teacher, Prof Michele Arslan, our friend Prof Oscar Sala, from Padua, Prof Vincenzo Ricci, from Verona, and all the colleagues coming from several hospitals in our various regions I would also like to extend a special welcome and thanks to Prof Vinko Kambič, from Ljubljana, and the numerous Jugoslav laryngologists who bring here their clinical experience and collegial friendship I am very sorry that Mr Henry Shaw M D , F R C S has been unable to leave London to participate in our meeting but he has kindly transmitted to us an ample summary of his contribution, for which I thank him very much Finally, we owe warm thanks to Prof Luigi Giarelli, our kind guest in this Institute who brings, together with his collaborators, an important contribution to our meeting

Introduction

The discussion at this round table symposium is not original, not new. During recent years several meetings and numerous papers (E Bocca et al , *Arch Ital ORL*, 1974, 11°, 4= Tumori, 1974, 60, 6) have been devoted to the precancerous lesions of the larynx but the problem is still complicated and obscure

The reasons for the unsatisfactory state of our knowledge concerning this topic are many first of all there is the problem of *nomenclature* resulting from the misuse of pathological and clinical terms by both Clinicians and Pathologists

Secondly there is the objectively complicated problem of *classification*, for which strictly reliable parameters are lacking and this leads to the existence of several such classificative models, which are difficult to compare with each other

Finally there are the clinically very difficult problems of prevention, prognosis, and follow up of the patients at risk

Of all these arguments I hope that mention, followed perhaps by discussion, will be made during our meeting

During these recent years, furthermore, two additional developments of laryngology have paradoxically conspired to increase our difficulties and perplexities in this particular field one is the important development of direct suspension microlaryngoscopy This procedure, by allowing a far more detailed examination of the larynx, has demonstrated how often abnormal conditions of the mucous membrane coexist with the principal one, clinically prominent, but perhaps entirely benign, which brought the patient to the Laryngologist This scattering and differing evolution of lesions requires multiple biopsies which will in turn render the prognosis and the treatment more difficult

The other is the development of conservative surgical techniques It is evident that the ascertainment of the coexistence of precancerous lesions in the same organ together with a limited, frankly neoplastic one, which could be treated by conservative surgery, is such as to influence the entire rationale of the treatment, especially from the prognostic point of view These new developments, on the other hand, place the role of radiotherapy in a different perspective, which, by itself, has greatly advanced in technique and instrumentation These are only a few of the problems which are involved in the title of today's meeting and which, I hope, will be discussed and analysed

Thank you

Observations of the Pathologist on Precancerous Lesions of the Larynx

Integrated with Histological Data and Quantitative Analysis of Nuclear DNA Content

L Giarelli, F Silvestri, G Antonutto and G Stanta

Institute of Pathology, University of Trieste Trieste Italy

It may be useful to remember, that the adult laryngeal mucosa appears to be composed of two distinct types of epithelium

1) pseudo stratified columnar ciliated epithelium with goblet cells, which covers a large part of the larynx,

2) stratified squamous non keratinized epithelium which covers the free border of the vocal cords, the lingual and part of the laryngeal surfaces of the epiglottis, and the medial border of the plicae aryepiglotticae

There is a rather abundant submucosa at the level of the vestibule as well as in the hypoglottic region The lamina propria is formed of collagenous and elastic fibres, and of a fundamental amorphous substance Comparing what we consider to be the classic structure in the adult, with that which has undergone the phenomena of aging, we must add that with age the stratified squamous epithelium is more extensive, this occurring at the expense of the columnar epithelium with goblet cells

These short notes contain a point of illuminating importance for the understanding of laryngeal carcinoma It is common knowledge that cancer of the larynx is, in almost all cases, a carcinoma of the squamous epithelium This characteristic places carcinoma of this region in a position of close proximity to that of other organs such as the esophagus, in which the reason for this particular aberration is unanimously ascribed to the stratified squamous epithelium which covers it in its entirety

We might also add that the carcinomas of both regions share the characteristic of being much more frequent in the male sex

From the aforesaid it appears evident how carcinoma of the larynx presupposes an almost compulsory condition the presence of stratified squamous non keratinized epithelium which is already present in normal subjects, or a fundamental modification of the columnar epithelium transforming it into a stratified squamous epithelium From this, lesions will subsequently develop on which the cancerous lesions will arise

Via this approach one immediately notes how the modifications of the laryngeal mucosa already confer a characteristic complexity to the clinical pictures which are frequent

Table I

A) Hyperplasia of the basal cells in the stratified epithelium or of those undifferentiated cells in reserve in the columnar epithelium
B) Squamous metaplasia (of the columnar epithelium)
C) Acanthosis and keratosis (of the squamous epithelium)
D) Dysplasia
   slight
   severe
E) Intra-epithelial carcinoma
F) Microinvasive carcinoma
G) Invasive carcinoma



Fig 1 Larynx hyperplasia of the basal cells of the epithelium (biop 4106/74) H&E ×50

ly met with This justifies also the vast number of terms commonly employed to indicate processes which in the final analysis turn out to be very similar This last and very widespread inconvenience renders the statistics and collections of material difficult to ratify, thus making it somewhat nebulous and difficult to identify the very points of reference which possess such a variable terminology

For the plethora of terms by which the laryngeal lesions are commonly distinguished there is no corresponding real difference in clinical picture This presupposes the possibility of simplifying the terminology so as to

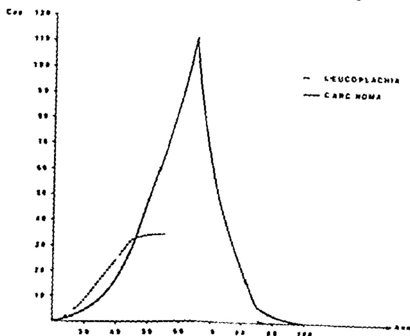


Fig 2 Incidence (number of cases) as a function of age for leukoplakia and carcinoma of the larynx respectively (in a total of 893 cases)



Fig 3 Slight precancerous dysplasia of the larynx (biop 3599/67) H&E ×50

give the Clinician a useful criterium for the necessary evaluation of the patient (Bocca et al , 1974)

As a whole, it is possible to recognize the existence of clinical pictures which, for the above mentioned reasons, probably exceed in number those known for the portio uterina Nonetheless it seems that a work of reordering, taking these well known clinical pictures as a point of reference, would afford certain advantages

Thus, the following table seems to group quite accurately the lesions which constitute each individual clinical picture (Table I)

The hyperplasia (of the basal cells in the stratified epithelium or of those undifferentiated cells in reserve in the columnar epithelium) includes those processes characterized by the simple proliferation of cells without the involvement of other modifications, and corresponds to the so-called simple hyperplasias (Fig 1)

However, when acanthosis appears, it would seem correct to include these lesions in the group of metaplasias (including the leukoplakias) Nonetheless, for this and perhaps *only* this group, the Pathologist has the impression that in the larynx the leukoplakia has a configuration which distinguishes it from that of other regions In this organ, more frequently than in others, the leukoplakia is associated in its morphology with atypia of the cells This can well justify the inclusion of leukoplakia of the larynx with the lesions of high evolutionary potential towards carcinoma which occur rather rarely in many other regions (Pizzetti 1974) (Fig 2)

For these reasons the difference between leukoplakia and dysplasia is not so clearcut as would appear at first observation

In the latter (dysplasia) the maturation of the elements is altered and keratinization occurs precociously

While in slight dysplasias (Fig 3) the stratification of the cells is still clearly present, it is changed in the severe dysplasias (Fig 4) because the altered maturation also results in an altered polarity of the cells themselves, with serious disorder of the stratification

In the severe dysplasias, some atypia can

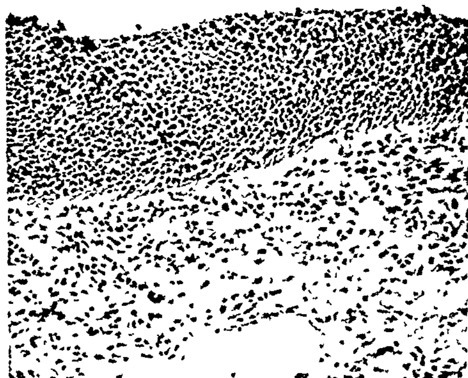


Fig 4 Severe precancerous dysplasia of the larynx (biop 11709/69) H&E, ×50

also occur in addition to hyperchromasia and the increased volume of the nucleus

At histochemical investigation, the *basement membrane* of the normal laryngeal mucosa is a structure composed of two elements a fundamental amorphous material around the basal pole of the cells, and a fibrillar argentaffin membrane laced in the same material The amorphous material is PAS-positive and is a complex polysaccharide

In hyperplasia and metaplasia of the laryngeal mucosa the basement membrane retains its morphology almost intact Only in dysplasia and especially in that which is more pronounced does one observe a reduction and disappearance of the amorphous material, and along with this the connective fibrils become more dense

Regions of disorganization of these fibrils are also found in the carcinoma even if initially, invasive (Trentini et al 1967 1970 1973) Taken in its complex the study of the behaviour of the basement membrane confers a certain character of homogeneity on all those forms which have in common the presence of cellular atypia Consequently these retain the easy possibility of evolving toward a neoplasia in the real sense The discord in opinions as already mentioned is maximum with regard to the leukoplakias, pachydermias and keratosis for which statistics indicate a possibility of a most variable evolution towards the carcinoma

Most probably this is to be ascribed to the diverse criteria employed in classifications, in which cellular atypias have not been suitably evaluated

What will be the destiny of a leukoplakia, and in particular, should it be endowed with properties placing it among the precancerous lesions of the larynx? This question, posed by the Clinician, cannot be answered with the same criteria valid for the other anatomical regions which are equally frequent sites of leukoplakia Instead we must point out that the heading "leukoplakia" is probably not sufficient to formulate a standard which we can follow for the purpose of reconstructing its evolution This is due to the variety of processes which are included under this heading



Fig 5 Intraepithelial carcinoma of the larynx (biop 11432/69) H&E ×100

Concerning dysplasias of medium and marked gravity, and moreover for intra epithelial can cer (Fig 5) no doubts should exist as to their capacity (although not obligate) to evolve toward infiltrative lesions This conviction is based not only on the fact that this is the case in most instances It is also derived from the frequent ascertainment of the coexistence of a laryngeal cancer apparently circumscribed by areas of mucosal dysplasia or even of intra epithelial carcinoma, which are placed in the contralateral specular regions

Our observations on this topic are signifi cant although not numerous We maintain, however that this rarity of assessment is not objective but rather tied to the fact that the number of larynges studied subsequent to ex tirpation is extremely few This is more true of the patients who have undergone a conservative operation and who are considered by the attending Physician who is not a Laryn gologist, as subjects no longer having any problems to bring to the Pathologist s attention because a precise diagnosis has already been made

By way of integration with the foregoing discussion, we deemed it necessary to correlate the histopathological study of our material with a histophotometric analysis

The variations of nuclear DNA have in fact constituted the basis of much research useful for a better understanding of the phenomena that underlie the basis of carcinogenesis

Materials and methods

There were examined three cases of simple leukoplakia (biop 15068/70, biop 4479/71, biop 2419/71), one case of leukoplakia associated with slight dysplasia (biop 14541/70), one case of leukoplakia associated with severe dysplasia (biop 6042/72) and one case of carcinoma in situ (biop 467/71)

All the tissue fragments sectioned were im mediately fixed in a 10% neutral formalin solution and subsequently fixed in paraffin From each block 5 μm thick slices were cut



Fig 6. Typical leukoplakia of the larynx (biop 15068/70) H&E; ×20

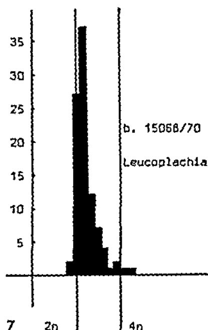


Fig 7 Histogram of the nuclear DNA content in typical leukoplakia (biop 15068/70).

with a microtome and subsequently stained with hematoxylin and eosin, and van Gieson.

Other slices, each 10 μm thick, were cut from each block and subsequently stained by the Feulgen reaction according to Stowell, using a hydrolysis time of 30 min with H-2 6-N at room temperature. The slices were mounted in Cargille® oil with 1.54 index of refraction. Determinations of the nuclear DNA content on the preparations thus obtained

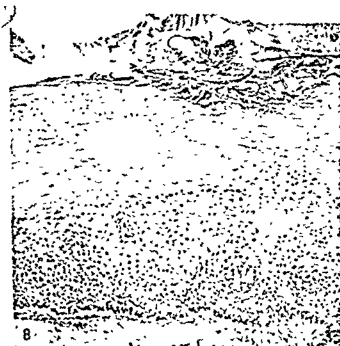


Fig 8 Typical leukoplakia of the larynx (biop 4479/71) H&E; ×16.

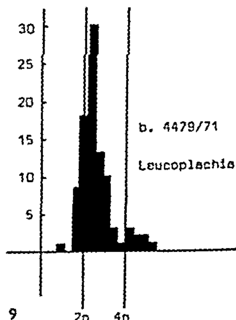


Fig 9 Histogram of the nuclear DNA content in typical leukoplakia (biop 4479/71)

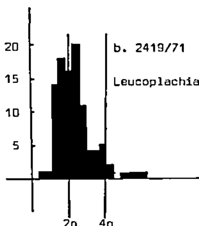


Fig 10 Histogram of the nuclear DNA content in leukoplakia of the larynx (biop 2429/71)

were made using a microspectrophotometer Leitz MPV-2

All the measurements were made according to the two-wave length method of Ornstein & Patau, which for our material was respectively equal to 575 nm (at the maximum of extinction) and at 510 nm (at half the maximum of extinction) The optic apparatus comprised an objective condenser (50/0 60 per MPV 2), an objective (NPL Oel 100/1 3) and a pair of eyepieces (Periplan G F 10XM) with graduated grid

To delimit the area of measurement, interchangeable diaphragms with fixed apertures were used

In each case 100 nuclei were measured on the epithelium Ten small lymphocytes were measured in each preparation as a control The evaluation of the data obtained by such measurements was made with an Olivetti 101 computer

The quantities of DNA thus determined were expressed in arbitrary units (A,u)

Results

With the histophotometric investigation, we can easily demonstrate how in a normal epithelium the modal classes are predominantly comprised of diploid and paradiploid cells in the G-1 phase (interphase) of the cell cycle (Avtandilou & Kazentseva, 1973)

In two cases of leukoplakia (biop 15068/70, biop 4479/71, Figs 6, 7, 8, 9) the images in the diagram do not vary much from those of a normal laryngeal epithelium In practice they do so only by the fact that the modal value of the histogram of the leukoplakia is slightly greater than that encountered in normal epithelium (in which it coincides with a value of 2 N) This would indicate a more conspicuous numerical presence of cells in the leukoplakic epithelium

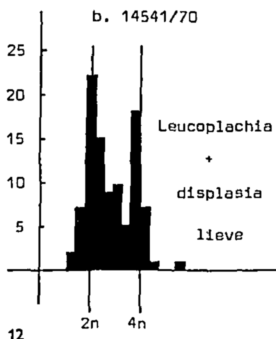
In the case of biopsy 2419/71 (Fig 10), the histogram in its general characteristics, mirrors the course described in the preceding diagrams with the exception that the modal peak has a slightly more "squat" appearance This tends to favour a more accentuated presence of cellular elements, "atypical for the DNA content", being either larger or smaller than the diploid value as well as greater than the tetraploid value

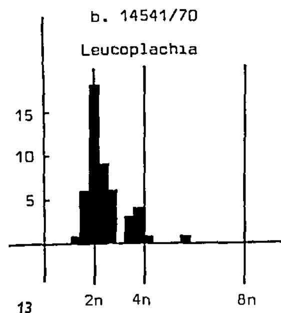
In the case of biopsy 14541/70 (figs 11, 12, 13, 14) diagnosed as slight dysplasia associated with leukoplakia, the histophotometric measurement was made separately for the keratinized part of the epithelium, and for the deeper part which had a slightly dysplastic aspect that occasionally tended to deepen into the submucosa The values obtained reflected the great difference existing between these two lesions One notices how the meas urement made on the part of the tissue with leukoplakia is substantially in agreement with that stated previously regarding this lesion

On the other hand, one notices how the dysplastic part (limited to a few discs having a papillary appearance, and constituted of a layer and a few overlying cells) reveals in its histogram a peak modal value equal to that of the tetraploid (4 N) This falls off abruptly to the right and more gradually to the left (although in a more discontinuous manner) until reaching low values slightly below 2 N

This picture clearly depicts the presence of an accentuated DNA synthesis, and the persistence of a longer G 2 period of cellular elements with DNA content equal to 4 N One should note, however, the absence of cells







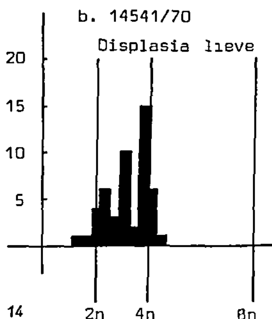


Fig 11 Larynx leukoplakia associated with a slight dysplasia of the mucosa (biop 14541/70) H&E, ×40

Fig 12 Histogram of the two lesions illustrated in Fig 11, considered collectively

Fig 13 Partial histogram referring to the leukoplakia illustrated in Fig 11

Fig 14 Partial histogram referring to the dysplasia illustrated in Fig 11

with a DNA content superior to the above-mentioned value, thus confirming the slight degree of dysplasia

Due to the proximity of the measured areas, we felt it necessary to add the two partial histograms, thereby obtaining a composite histogram which sums up the characteristics of the two lesions

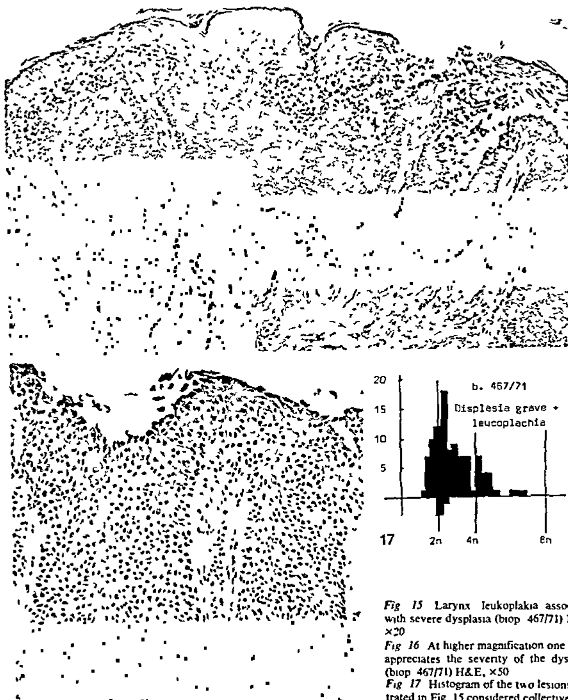


Fig 15 Larynx leukoplakia associated with severe dysplasia (biop 467/71) H&E ×20

Fig 16 At higher magnification one better appreciates the severity of the dysplasia (biop 467/71) H&E, ×50

Fig 17 Histogram of the two lesions illustrated in Fig 15 considered collectively

From the above considerations a better analysis of biopsy 467/71 (Figs 15, 16, 17) has been obtained in which there is a grave dysplasia associated with the leukoplakia In this case one notices the presence of the characteristic peak of the leukoplakia the histogram decreases to the right more slowly than in the cases of simple leukoplakia In particular one notices the presence of conspicuous number of cells included between the values 2 N and 4 N This indicates an increased presence of tetraploid cells, and other cells with a DNA content



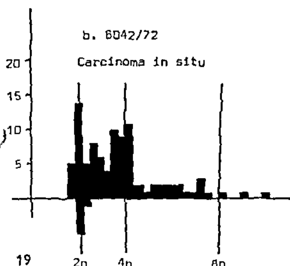


Fig 18 Larynx carcinoma in situ of the mucosa (biop 6042/72) H&E ×50

Fig 19 Histogram of the nuclear DNA content of the carcinoma in situ illustrated in Fig 18

higher than this value which by interpolation can be evaluated as equal to 6 N

Finally there remains the examination of the last lesion considered, i e the carcinoma in situ

The analysis of the histogram of biopsy 6042/72 (Figs 18, 19) reveals the presence of a thin diploid peak One should also note the presence of a discrete number of cells in the region between 2 N and 4 N which contribute to the formation of a squat tetraploid peak This peak declines abruptly to the right where it continues in a plateau which bypasses the value 8 N and which reveals a slight tendency to form a small peak in a paraoctaploid region

The persistence of tetraploid cells and the presence of a plateau reveal two facts On the one hand they demonstrate the subversion of a normal cell cycle (increased synthesis and persistence of tetraploid cells by the lengthening of the G-2 period of the cell cycle) On the other hand they reflect the tendency toward completely atypical proliferation of the cells (elements beyond the tetraploid value) due to the abnormal content of nuclear DNA

Conclusions

It is the pathologist's impression that a multiregional participation by the laryngeal mucosa in a dysplastic process is not a rare occurrence and it is conceptually valid in that the elements responsible and clearly identified act throughout the mucosa Concerning our experience we might add something more We suggest the



Fig 20 Larynx microinvasive carcinoma (biop 3907/71) H&E, ×20

hypothesis that the same factor capable of evoking the damage to the laryngeal mucosa thus rendering it capable of occasioning a subsequent carcinoma, will reveal its damage elsewhere It will do so not only in multiple regions of the larynx, but also in other regions farther down the respiratory pathways, especially at the bronchiole level

This is the significance that we believe possible to infer from the observation which we made in our Department Of the 409 pulmonary carcinomas studied at autopsy, several (41) resulted as being associated with malignant tumours of other viscera Undoubtedly, the larynx is the viscera which is by far the most frequently present in this association In fact, from our review it occurs in 15 observations

We note with considerable astonishment how many authors are surprised by the fact that the group of carcinomas in situ, the microinvasive cancers (Fig 20), and those of the m of Bowen, are omitted from the chapter on carcinomas Their argument in support of the inclusion is that the cancers in situ nonetheless deal with cancers, because statistics are in

agreement with pointing out the definite evolution of the lesion towards the infiltrative carcinoma (even if within the space of 5–10 years).

However, we object that precisely herein lies the interest in identifying these forms with the best possible objective assessment. With this, the clinician, well convinced of what will follow, has a sufficiently long time span in which to interrupt a course which is otherwise judged fatal.

This is why we have proposed that both groups of lesions could be examined in a single discussion—that is, those which prepare the terrain for the carcinoma and those that represent the early stages.

The research of Sirtori (1963, 1964) and of Kleinsasser which represents a fundamental stage in the study of laryngeal carcinoma, is in harmony with these conclusions. In Sirtori's opinion, the major part of the so-called relapses occurs at the level of the larynx, because in this region more so than elsewhere, the carcinoma has a progressive characteristic. Kleinsasser is substantially in agreement with this statement and affirms that sooner or later the carcinoma in situ evolves towards the carcinoma in 90% of the observations

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Macro-microscopical Findings and Prognosis of Hyperplastic Aberrations of the Laryngeal Mucous Membrane

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The study of hyperplastic aberrations of the laryngeal mucous membrane is extremely important from both a practical and a theoretical point of view, especially on account of the relationships existing between these changes and the precancerous and really cancerous lesions of the larynx The medical literature is indeed very rich with reports of pathological and clinical research which try to coordinate and define these changes by means of the histological picture, and so determine their prognosis

The basis for the classification of laryngeal hyperplastic aberrations is the approaching of the histological picture of these changes to the histological picture of cancer However, the histopathological picture, which is immediate, and the biological qualities of the hyperplastic aberrations, are not always in mutual agreement For this reason a great many difficulties are encountered, especially when we try to define the aberrations pertaining to the group of the so-called precancerous lesions

The precancerous stage should be a determined quality of the tissue from which, sooner or later, a malignancy may develop

The key question in the general problem of cancer is *under what conditions* and *when* will the pathological alteration of the tissues—the so-called precancerous conditions, i e the pathological aberrations which do not have all the known histological, biological and clinical characteristics of cancer—turn to real malignancy

The difficulties appear already in the terminology, various authors describe identical clinical changes by different names, such as keratosis, pachydermia, leukoplakia or hyperkeratosis, which often causes a great deal of confusion

The histological picture which should be taken as characteristic of the tendency of malignant degeneration of the tissue is not the same for all authors

In the literature we have found 14 different classifications of epithelial hyperplasia Among them many are obviously similar—we could even say that they are identical

For this reason the adoption of an internationally agreed classification based on specifically defined histological characteristics is really necessary The clinical (and also the microlaryngoscopic) criteria of classification have no real value, especially from a prognostic point of view, as no definite diagnosis can be responsibly based on these data alone although an intimate collaboration between the laryngologist and the pathologist certainly give the diagnosis a definite statistical improvement of the degree of correctness

We shall discuss only the second classification of Kleinsasser (1962) which has been accepted by many authors (1) hyperplasia simplex of the epithelium, (2) hyperplasia of the epithelium with individual cellular atypia, (3) precancerous epithelial lesion or "carcinoma in situ".

Kleinsasser's third group, and the analogous

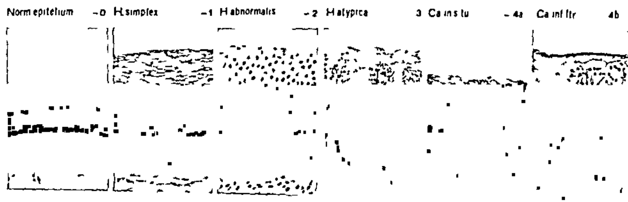


Fig 1

classifications suggested by other authors, corresponds to the non-invasive atypical epithelium which shows all the changes of epithelial cells and cellular stromata found in carcinoma, with the only difference that the invasive growth is not present

Our opinion is—taking it into account in our own classification—that a "carcinoma in situ" is not a precancerous stage of the disease in the strict meaning of the word but a real carcinoma

No one can maintain that in a "carcinoma in situ" the basal membrane is intact Apparently this one is not damaged because its changes are not appreciable by today's diagnostic methods At first, indeed, these changes are very likely only of a biochemical nature, and most likely a big step forward will be made with the electron microscope and the histochemical methods of analysis

Also in the booklet ' The TNM Classification of Malignant Tumours' , issued in 1968 by the UICC and prepared by a Committee for the TNM classification of tumours, the "carcinoma in situ' or the so-called pre-invasive carcinoma, is classified with the really malignant tumours and not with precancerosis (precancerous stage)

Most authors classify keratosis as a significant factor in cancerization, though others believe that this is not essential

Therefore, in the light of all that is mentioned above, we must not be astonished if the very identification of the cytohistological characteristics forming a transition from laryngeal hyperplasia to carcinoma—known under the name of precancerosis—is one of the most serious problems with which numerous Laryngologists, Histologists and Oncologists are grappling with

We have classified the hyperplastic aberrations of our clinical material (in the last 10 years we have made over 2500 biopsies in which the laryngeal mucous membrane was pathologically changed) into three groups regarding the histological picture

(1) "*Hyperplasia simplex*",
the simple hyperplasia

The epithelium is thicker, chiefly due to a thickening of the prickle-cell layer The layer of basal cells is unchanged

(2) "*Hyperplasia abnormalis*",
the abnormal hyperplasia

The epithelium is thicker on account of "basalization" the cells of the basal type extend up to the middle of the epithelial layer There are no pathological atypies anywhere

Both these forms typically represent the hyperplastic processes of the epithelium of the laryngeal mucous membrane which regularly do not turn to malignancy and are of a reversible nature

(3) "*Hyperplasia atypica*",
(atypical hyperplasia)

This group includes atypies until "carcinoma in situ" The atypia occupies all the epithe-

lium The cells of the entire hyperplastic epithelial layer take on the form of the basal cells The nuclei are hyperchromatic, slightly polymorphic, scattered mitosis may be observed, which is partially atypical

(4) *Carcinoma*

(*a*) "*Carcinoma in situ*", the pre-invasive carcinoma The basal membrane is apparently preserved There is a heavy reaction of the mesenchyme with presence of immuno-competent cells, with all the signs of cellular and nuclear atypia macronucleosis (anisonucleosis), cellular atypia, pathological mitosis appear up to the surface of the epithelium There are also signs of cellular dissociation

(*b*) *Carcinoma invasivum* Histological characteristics are already well known to us

Only the third group is reckoned, by us, among the precancerous lesions of the larynx

The percentage of malignancies within these changes is relatively high From 80 cases belonging to this group after our classification, 20 cases became malignant within a period of 1–3 years From the first and the second group no aberrations became malignant

In this classification we have not taken into account the keratinization, because, after our experience, we came to the conclusion that this sort of lesion is not particularly significant in the process of malignancy

We adhered closely to the principles that the process of cancerization is the consequence of two developments, namely (1) the change in epithelium, and (2) the reaction of mesenchyme which represents the common response of the organism

(Ad 1) Among all the pathological processes appearing in the epithelium, the hyperplasia is the most significant

The hyperplasia suggests that the specific function of the cells is subordinated to the growth function From our own actual knowledge we can state only incompletely which cellular changes arises during this condition

Taking this into consideration, keratosis can be considered as of only secondary significance Electronmicroscopy showed that keratin proceeds from tonofibril epithelial cells as a phenomenon of dystrophy We cannot say anything else about the prognosis of pathological epithelial changes by considering the phenomenon of keratosis alone keratinization means only a certain degree of degeneration

The phenomenon of *dyskeratosis* has another meaning this one demonstrates a certain dysfunction, i e a "side" function of the cells and reveals an increased potential of division

(Ad 2) Reaction of the organism The response of the organism is demonstrated by the infiltration of the Reinke space by mastocytes, plasmacytes, and by the increased quantity of acid mucopolysaccharides (hialuronic and chondroitin sulphurous acids) in the mesenchyme

If these elements are not found in the mesenchyme, then it is conceivable that the reactivity of the organism has not yet changed

Unfortunately, we have no firm histological criterium which can help us to diagnose reliably the precancerous stage in the sense that this term signifies

Lesions which look essentially alike from a macroscopic or microscopic point of view, can change to malignancy or stop in their evolution They may regress partially or entirely, as a consequence of external or internal influences of which we know very little as yet

Precancerous Lesions of the Larynx

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These lesions are few in number and usually related to chronic irritation of some type Although often persistent, only a small minority will eventually undergo malignant change

Classification

(1) Chronic diffuse hypertrophic laryngitis of adults

(2) Keratosis laryngis (leukoplakia of the vocal cords)

The latter may theoretically be subdivided into (*a*) superficial dysplasia without atypia, (*b*) marked dysplasia, usually with atypia, (*c*) carcinoma in situ

In addition, there is the clinical classification into "white" or "red" keratotic lesions, the latter usually being the more advanced histologically

There are two main difficulties in classifying this disorder Firstly, Pathologists vary greatly in their individual interpretation of any biopsy specimen Secondly, in view of the wide variation of change often present in any one lesion, the limitations of biopsy are obvious

(3) Occasionally malignant change may occur *in the rare* laryngeal papillomas of adults

Diagnosis

This must be established by accurate clinical observation via indirect and often direct laryngoscopy The latter method is today greatly facilitated by the use of microlaryngoscopy under general anaesthesia, according to the technique of Kleinsasser

A valuable aid in the selection of a biopsy site is the use of 1% Toluidine Blue dye lightly painted over the area and then sponged off with saline Biopsy is then made in the area taking up the dye most strongly. Cytology examination of smears is not of much value

Treatment

As a result of difficulties encountered in obtaining a reliable histological picture of the lesion it is better to treat it on clinical evidence and according to the known responses to treatment modalities

These lesions are precancerous only if persistent over a period of years and then only in a *minority* of cases Estimates vary from 5–15% showing eventual malignant change

It is also known that irradiation is required in full doses to eliminate keratosis (i e 6000 rads), which then often recurs

If there is then a later malignant change, irradiation cannot be used again These facts would seem to contra-indicate its use It should never be given for diffuse hypertrophic laryngitis In view of this, two alternatives are available (1) Removal of all possible sources of irritation (tobacco, alcohol, etc) and voice rest with regular observation over a period *of months is the first step (2) If the* lesion is still persistent and localized, surgical removal by stripping of the vocal cord in cases of keratosis may be undertaken using a microlaryngoscopic technique Another useful modern technique is the careful use of the cryoprobe

In cases of diffuse hypertrophic laryngitis,

no surgical treatment is advised Avoidance of irritation and medical measures are the rule

Should any definite diagnosis of *carcinoma* either *in situ* or *invasive*, be made at biopsy, therapeutic irradiation to full dose can then be given, usually with success in a localized lesion Where the carcinoma in situ lesion is small it is also permissible to use a stripping technique in the first instance with irradiation in reserve for any recurrence

In the rare case of malignant change in a laryngeal papilloma, irradiation, and partial or even total laryngectomy may be used, depending upon the extent of the disease For this reason those papillomas are best removed by surgical stripping or partial laryngectomy at an early stage

Prognosis

The response to treatment of these lesions is often disappointing as the main cause—chronic irritation or vocal stress—cannot always be avoided Therefore patients must be kept under regular review at 3–6 months intervals This will also apply even with apparently successful treatment of a localized lesion, in view of the possibilities of recurrence

Contributors

Prof. Michele Arslan
Emeritus of Otorhinolaryngology,
University of Padua Italy

First of all I would like to congratulate Professor Bosatra for the organization of this Symposium dealing with an argument which undoubtedly is not new but which is undergoing considerable conceptual and practical evolution and which has puzzled us since the beginning of Laryngology

Indeed, from several points of view, laryngeal lesions which are referred to us with the label "precancerous" are very often most intriguing as regards treatment and prognosis

The elements of uncertainty are more numerous than in cases of real cancer

Taking into account the paucity of the therapeutic tools which we have at our disposal for the treatment of precancerous lesions I hope that during this Symposium an analysis and discussion will be devoted to the role of radiotherapy During our long practice we have used it satisfactorily on several occasions Furthermore this method has undergone very important conceptual and technical improvements during recent years

Prof. V. Ricci
2nd Department of Otorhinolaryngology
University of Padua Centre of Verona Italy

There is no doubt that the precancerous lesions of the larynx represent a challenge to the Laryngologist from a diagnostic, and especially from a prognostic and therapeutic point of view

I fully agree, therefore, with the purposes of this meeting as organized by Prof Bosatra, in which the knowledge and the experiences of the Laryngologist, of the Pathologist and of the Radiotherapist are mutually confronted

The main problem, as it arises also from today's lectures, and I refer especially to the beautifully documented histological and histochemical research of Prof Giarelli and collaborators, is the prognostic one the destiny of the leukoplastic or hyperplastic lesions of the larynx

We all agree that for methodological and clinical reasons it is practically impossible to obtain reliable data on the malignant evolution of these lesions from the statistical values of the various A A , which range from 3 to 70%

I believe that a better knowledge on the *immune reactions* that develop at the level of these lesions will give us very useful information as to their evolution and treatment

As a consequence of some studies on the immune reactions which are promoted by a laryngeal cancer in the guest organism (Ricci et al , 1967), I am convinced that a strict link does exist between the two conditions and that the appearance of auto antibodies against the laryngeal epithelial cells represents a guide to the further evolution of the lesion

The peculiar hyperergic condition of the mucosal membranes of the upper respiratory and digestive pathways, which differ in a definite way from that of other territories, has been experimentally and clinically demonstrated in our Department

The mucous membranes of these regions, which represent the first barrier between the external world and the organism, are a "shock organ" well able to develop a high degree of tissue immunity, and this peculiar characteristic is further enhanced by frequent episodes of local phlogosis, often of sub chronic course

From this point of view, and also from the pathological, clinical and statistical data of Bocca et al , it is conceivable to classify the leukoplasic lesions of the larynx under various headings according not only to their histologi

cal appearance but also to their tendency to develop different degrees of histo-anticorporeal immunity against the epithelium

We can identify, therefore

(1) Leukoplasic lesions of 1st degree usually small in size, arising on a larynx not sub ject to an obvious irritative agent, and therefore with a slight concomitant phlogistic com ponent These lesions are not such as to promote an immunological response and therefore have a very reduced tendency to malignancy

(2) Leukoplasic lesions arising over a chronic phlogistical base as a consequence of well defined *irritation* (traumatic lesions) the abnormal immunological response is possible and the evolution towards malignancy much more frequent (Leukoplasies of 2nd degree)

(3) Leukoplasic lesions in which although initial, the malignant evolution is already pres ent in these cases it has been possible to dem onstrate the presence of specific agglutinated antineoplastic antibodies in the patients' serum, with rather elevated titres (Leukoplasies of 3rd degree=cancer in situ)

In conclusion, I believe that together with the microlaryngoscopical, histopathological and statistical methods of examination, the immunobiological data can be added to inte grate our knowledge of this type of lesion, and consequently establish a better therapeutic guide

Prof. O Sala

Department of Otolaryngology
University of Padua
Padua Italy

Provided that the study and correct classifica tion of a precancerous lesion of the larynx are the result of constant cooperation between the Histopathologist and the Laryngologist, so as to greatly reduce the possibility of error, I would like to analyse three different condi tions which we all have encountered, viz (1) laryngeal lesions in which the microlaryngeal pattern prevails over the histopathological one, (2) lesions in which there is agreement between the clinical laryngeal pattern and the histopathological one, and (3) lesions about which the Histopathologist and the Laryngologist may lead each other into error

The first condition is now particularly common, owing to microlaryngoscopy Careful examination of the larynx in chronic laryngitis discloses some areas where the surface epithelium is irregular, thickened and whitish in appearance, especially where the mechanical friction is stronger, such as the anterior part of the true vocal cords and also the false vocal cords when they are hypertrophic Though the fragment to be examined is large enough, the Histopathologist may note only thickening of the epithelium displaying a well preserved base membrane and a moderate infiltration of the stroma a completely non specific pattern, even though clinically the patient shows clear signs of a chronic disease (dysphonia, etc)

The second condition is the most common and the most interesting from the clinical point of view

The larynx shows a thickened, white area, rising over the surrounding epithelium, usually located on the free margin of the true vocal cord between the anterior and the middle thirds The border of the lesion stands out distinctly against the surrounding mucosa which displays a normal appearance, without signs of congestion and oedema

Histopathology confirms that the epithelium is fairly active, particularly in the superficial layers, with an excess of keratin production The basal layers are well preserved and the base membrane itself is intact A moderate infiltration of the connective stroma is usually noted In other words, there is agreement between the clinical aspect and the histopathological pattern—they both confirm a well delimited lesion of the laryngeal epithelium

This patient is to be checked up periodically Later he may show some congestion and oedema surrounding the white patch The Laryngologist must then provide the Histo-

pathologist with a sizable biopsy which should include the epithelium, the base membrane and the underlying stroma, so as to give an overall view of the laryngeal lesion and of the tissue reaction This biopsy, if positive, shows an active basal epithelium, though mitoses are not atypical The base membrane is still well preserved though its aspect is not as even as usual The connective stroma may display two distinct reactions—either there is a marked inflammatory infiltration, mainly of plasma cells, which indicates the body's defensive reaction, or the stroma may appear almost unaffected, exhibiting only oedema and a few lymphocytes, which indicate a poor local reaction, this against a histopathological pattern revealing an epithelial activity which, if not pathologic, is certainly not normal

These patients need periodical follow up, as the neoplastic disease may develop suddenly, apparently without cause—sometimes after a common acute virus disease (influenza)

The third condition is undoubtedly the most important, also for practical purposes, and it is represented by the *verrucose squamous carcinoma of the larynx* Recently, this entity has been histologically and clinically defined (see *Ferlito et al, 1975) From our experience* (during the years 1968–1975), we have learnt that this lesion is not as uncommon as one might believe Its supposed rarity may well be due to the fact that the lesion is often unrecognized, whereas it may develop also in other organs

The larynx (particularly the true vocal cords) usually shows an exophytic, whitish vegetation protruding into the glottis The area of implantation is small when compared with the visible exophytic mass The mobility of the cord is always preserved even when the lesion almost completely covers the cord This fact strikes the clinician when he first examines the larynx

A biopsy fragment can be easily removed and the Laryngologist believes that he has provided the Pathologist with a significant sample of the lesion In fact he has eradicated only the most superficial part of it, consisting of abundant keratin arranged along solid cords of well-preserved epithelium displaying all stages of physiological maturity

The negative biopsy report of such a sizable fragment, coupled to a clinical pattern only partly suspicious (mobility of the cord, mucosa neither inflamed nor oedematous around the exophytic lesion, etc) mislead the Laryngologist into believing that the lesion is benign

Later on the patient will be back to consult with the Laryngologist because the lesion has recurred and is even larger It almost fills the laryngeal lumen and the mobility of the cord is reduced If a biopsy fragment of adequate size is then removed (it should include the basal layer, the base membrane and the underlying stroma) it will show atypical mitoses in the deeper layers of the epithelium and a marked inflammatory infiltration of the connective stroma (plasma cells, small lymphocytes, etc) Inflammatory infiltration means that a strong defensive reaction is taking place in the healthy tissue, but it also reveals that the neoplastic lesion is vigorously expanding, though disguised as an exophytic tumour mass

If this condition occurs, the Laryngologist is led to believe that the existing laryngeal lesion has in the meantime become malignant but as a matter of fact, the carcinoma had always been present and would have been detected immediately if the biopsy fragment removed had been adequate For this reason, the study of the DNA content of neoplastic cells (Ferlito et al 1975) is particularly significant

The Histopathologist and the Laryngologist lead each other into this error The verrucose squamous cell carcinoma is indeed the least malignant among the malignant tumours of the larynx, because of the body's marked defensive reaction, the slow infiltrative development and the exophytic growth which makes it easier to establish an early diagnosis Minor surgery is indicated in this case, provided it is technically possible Radiotherapy, on the contrary must be avoided because it has an immunosuppressive effect which would cancel

the marked cellular immune response around the tumour In some cases, radiation may induce anaplasia

Metastases are usually not detected in cervical lymph nodes and therefore prophylactic neck dissection not only is not needed but is contra-indicated because the lymph nodes are the site of production of a useful local and general immune response

This experience has convinced me that the TNM classification of tumours (of all neoplasms and in particular of those of the larynx) is to be considered out of date because it takes into account only two factors, viz , site and extent of the tumour, while it disregards other factors which are much more important, among them the tumour–host relation

Indeed, a critical review at 5 years of a series of 104 patients with laryngeal carcinoma who had been either operated on or treated with radiation therapy in our Department during the year 1968, allows us to state that prognosis was poorer for those patients with poorly differentiated carcinoma and a minimal cellular immune reaction around the tumour, while the best prognosis was for the patients with well differentiated carcinoma and marked cellular immune reaction (Sala & Ferlito, 1975), irrespective of the therapeutic program adopted

I have thought it advisable to draw attention to such a new, critical approach to tumour pathology In the light of these new acquisitions, precancerous laryngeal lesions always need close attention and should be studied taking into account the tumour–host relation

Prof. A. Torretta

Head Radiotherapy Department
Ospedale Maggiore Trieste Italy

It is well known that considerable disagreement still exists concerning the treatment of laryngeal precancerous lesions this disagreement arises at least partially, from the statistical discrepancies concerning the frequency of the malignant evolution of such lesions

In this field, radiotherapy, especially since the advent of telecobalt therapy, has gained remarkably in importance as it has proved to be very efficacious in the treatment of some among these diseases

To define the therapeutic problem still better, I will mention some statistical data on the evolution of leukoplakias and cordal pachydermias

MacGavrin, Bauer & Ogura have found only three cases of evolution into carcinoma out of 84 cordal precancerous lesions, Gabriel & Jones, one case out of 18 Putney & O Keefe, on the contrary, found 57 epitheliomas out of 125 cases of laryngeal keratosis, out of the 68 hystologically benign cases 27 changed into carcinoma (40%), in periods varying between 6 months and 5 years

Norris & Peale, out of 85 leukoplasia cases, found 11 cases (13%) of epitheliomatous evolution, among which 4 were cancer in situ, 5 invasive cancer and 2 with doubtful invasion Lepage quotes very remarkable data about the totality of cases referred to in the literature up till 1965 during a 10 year observation period, keratosis without cellular atypias showed 1% of changes into cancer, those with atypias 14 7%, among them 6 2% into epitheliomas in situ and 8% into invasive epitheliomas

By comparing these concise data we may conclude that these lesions, which really can be considered borderline lesions, have to be considered very carefully either from a diagnostic or a therapeutic point of view

In fact, very often there are considerable doubts towards bioptic responses, not concerning the interpretation of the histological specimens, but for the possibility of finding neoplastic and dysplastic areas near the areas positive for simple pachydermia, or for the existence of cases in which multiple biopsies give a contradictory diagnosis of simple hyperplasia in the vegetative area and of epithelioma in the leukoplasic area

It is well known that only in the *simple pachydermia* are cellular atypias hardly ever present, on the contrary, they are frequent in

the *keratotic papillomata* and in the *leukoplakias* Many people consider leukoplakia not only to be a lesion fated to evolve with a high statistical probability toward cancerization, but already quite a starting stage of a malignant neoformation

Along the therapeutic lines suggested by Fletcher, we think that only simple pachydermia, especially if it has a limited spread, should not be submitted to radiotherapy

The keratotic papilloma and the leukoplasia, especially when they are substantially thickened, irregular and vegetative, must, on the contrary, be submitted to telecobalt therapy, according to very radical criteria, by using doses of 6000 rad focus (average), over a 5–6 week period

It is possible to reach even the 7000 rad level, according to the volume target area, by using very small fields, generally laterolateral For such therapy the treatment must last 5 to 6 weeks, at least In practice, by using telecobalt therapy, cartilaginous necrosis hardly ever occurs

By such a method (and Fletcher is a very firm supporter of this) such lesions recover in 90% of cases, without noteworthy scars In the relapses it will be possible to apply surgery (cordectomy, hemilaryngectomy) without local complications arising from the previous radiation

Prof. Andrea Bosatra

Director Ear Nose and Throat Clinic
University of Trieste Trieste Italy

My contribution to this Symposium will be limited to the role of microlaryngoscopy in the assessment of hyperplastic and precancerous lesions of the larynx

During the years 1969–1974, 113 cases have been observed affected by this type of lesion in 89 they were multifocal or diffuse in 60 cases the lesions were only hyperplastic or precancerous, in 15 cases there was an associated real cancer, in 7 cases there was an intra-epithelial cancer, in 7 other cases there was an intra-epithelial and real evolutionary cancer

In most instances the associated lesions have been discovered only during microlaryngoscopy and in some cases the toluidine staining of the mucosa represented a useful guide to the biopsy

Our method of performing this examination without intubation (and in full safety) certainly represents an advantage when seeking the various scattered lesions In any case this is an essential element when establishing the prognosis and planning the therapy, especially when, because of the existence of a real cancerous lesion, conservative surgical treatment is selected

Indeed this type of surgery bears in itself the risk of leaving areas of mucosa prone to future malignant evolution

This problem must also be considered in the evaluation of the hyperplastic cases in which a real cancer has developed during a subsequent epoch 10 out of 84 of our cases of simple hyperplastic or precancerous lesion (11 88%) evolved into real cancer within 6 to 30 months (average 18 3 months)

Because of the site of the plurifocal lesions, some of these cases, treated by conservative surgery, could have been classified as recurrencies

Nevertheless, the more complete information given by microlaryngoscopy is a fundamental element in the management of this pathology, even reducing those discrepancies between clinical appearance and pathological finding, mentioned by Sala

Our personal experience indicates that the period of greater risk of cancerous evolution even when all prophylactic measures have been adopted, lies within the period 6 months to 2½ years from the first diagnosis A more precocious cancerous evolution should, in our opinion be considered as an indication of a real cancer lesion (although minimal) arising since the first examination

Discussion

V Kambič Following my experience, I believe that a precancerous lesion should not be treated by radiotherapy In this case the cells are fully developed and not radiosensible Furthermore there is the possibility of promoting an evolution towards malignancy I believe, therefore, that the only active treatment of precancerous lesions is represented by "decortication" The opposite is true when we are dealing with cancer "in situ", or with carcinomata in stages T1, T2, in which case 90–95% of recoveries can be achieved by radiotherapy *Also from this point of view, the difference* is emphasized between precancerous lesion and cancer "in situ"

A Torretta I only wish to make it clear that the only lesions to be treated are those showing cellular atypia As for the results to be expected, I underline that a full dosage must be administered The treatment must not be stopped at 4000 rads, but protracted up to 5000 at least

V Kambič From a radiotherapy point of view, too, the necessity of clear cut terminology arises, with a precise distinction of the precancerous lesion (in which only a few exceptional atypia can be observed) from cancer "in situ"

A Bosatra I think we ought to kindly ask to Prof Giarelli to define this concept, which can be a rather subtle one

L Giarelli There is no doubt that an intra epithelial carcinoma is a real cancer, but I think that the specification *intra epithelial* is not meaningless to the Laryngologist I believe, indeed that the intra epithelial carcinoma has a slower evolution towards the infiltrative carcinoma allowing less precipitous clinical decisions regarding treatment

As regards the precancerous lesions, I think that the diagnosis must be based essentially on examination of the cells Possibly because we include within the term leukoplakia (or the like) of the larynx lesions basically different from each other the point is that these laryngeal lesions turn to malignancy much more easily than those of other sites for instance in the oral cavity (which by the way are much more frequent) It is possible that within the leukoplastic lesions of the larynx a much wider range is comprised of cellular abnormalities of determinant value These abnormalities must be evaluated and underlined to the Clinician it is assumed that only a very small percentage of cellular atypia can return to normality if we can stop the promoting factors Likewise, a frank inflammatory infiltration can allow a better prognosis I do not think that, at the moment, we Pathologists should give any further advice to the Laryngologists

A Bosatra Thank you, Prof Giarelli I see that another Pathologist wants to comment on this problem

A Ferlito (Department of Otolaryngology, University of Padua, Padua, Italy) Precancerous lesions of the larynx lend themselves to some general considerations First of all, it is necessary to underline that the definition "precancerous lesion" must be used to define various lesions, surely not neoplastic in nature, from which a carcinoma may or may not develop From Mr Shaw's and Prof Kambič's address we hear that "carcinoma in situ" is arbitrarily considered by some authors as a "precancerous" lesion We agree that "carcinoma in situ" is of course a malignant lesion, which unlike common carcinoma remains temporarily limited to the surface epithelium without going deeper than the basement membrane and without infiltrating the mucosal connective stroma There are two synonyms for such a lesion, viz pre-invasive carcinoma

alcohol, also through the effect of the liver dysfunction, vocal abuse) must be firmly prescribed

The local "stripping" of the mucosa under microlaryngoscopy can be very effective

Radiotherapy must be undertaken when the degree of cellular atypia shifts the diagnosis toward a real cancerous lesion

A Bosatra

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THE TRACHEA
and
CUFF-INDUCED TRACHEAL INJURY

An experimental study on causative factors and prevention

By

ULF NORDIN

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An experimental study on causative factors and prevention

By

Ulf Nordin

Department of Otolaryngology University Hospital Uppsala Sweden

This dissertation is based on the following papers, which will be referred to by their Roman numerals

I Nordin, U, Engström, B, Jansson, B & Lindholm, C E 1977 Surface structure and vascular anatomy of the tracheal wall under normal conditions and after intubation *Acta Otolaryngol* (Stockh) *Suppl 345*

II Nordin, U, Engström, B & Lindholm, C E 1977 Surface structure of the tracheal wall after different durations of intubation *Acta Otolaryngol* (Stockh) *Suppl 345*

III Nordin, U & Lindholm, C E 1977 The Vessels of the Rabbit Trachea and Ischemia Caused by Cuff Pressure *Arch Oto Rhino Laryngol* 215, 11

IV Nordin, U, Kallskog, Ö, Lindholm, C E & Wolgast, M 1977 Transvascular fluid exchange in the tracheal mucosa Accepted for publication in *Microvasc Res*

V Nordin, U, Lindholm, C E & Wolgast, M 1977 Blood Flow in the Rabbit Tracheal Mucosa under Normal Conditions and under the Influence of Tracheal Intubation *Acta Anaesthesiol Scand 21, 81*

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An experimental study on causative factors and prevention By Ulf Nordin

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Surface structure and vascular anatomy of the tracheal wall under normal conditions and after intubation

By U Nordin B Engstrom B Jansson & C E Lindholm

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CHAPTER 3

Surface structure of the tracheal wall after different durations of intubation.

By U Nordin, B Engström, & C E Lindholm

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Introduction

Tracheal intubation has a long and interesting history and is still an indispensable part of modern medical care

When Trendelenburg (1871) described the first tracheostomy cannula supplied with an inflatable cuff he introduced a method of producing a seal between the cannula and the tracheal wall which, in principle, is still used today Trendelenburg's cuff was small and thick walled, a typical representative of the so called *low volume cuff** Some years later Eisenmenger (1893) introduced the first *high volume cuff* and brought an improvement not only in respect to the cuff size In addition, he supplied the cuff with a large pilot balloon which was to signal and possibly also limit the intracuff pressure Eisenmenger was probably not fully aware of the true significance of the cuff volume—at least he published no analysis of the practical difference between a high volume and a low volume cuff Wilms (1904) described a tube supplied with a high volume cuff, in which holes had been made in the tube wall within the cuff area so that the pressure in the airway should automatically inflate the cuff The system was unsatisfactory however, as the holes easily became blocked with mucus This device was reintroduced by Mushin (1943)

The first analysis of the practical difference between the low and high volume cuff was reported by Grimm & Knight (1943) They recommended the use of a high volume cuff with a sufficiently large volume to allow it to fill the lumen of the trachea without the cuff wall being stretched Concerning the low volume cuff, they state,

"The heavy walls require considerable air pressure to expand these small cuffs to a volume sufficient to fill the trachea Since tracheae vary in size, it follows that an unknown amount of pressure is required to fill the trachea without applying any pressure to the mucosa Therefore, in such a cuff, it is impossible to measure the pressure applied to the tracheal mucosa This is of paramount importance since an undue amount of pressure causes either trauma or ischemia which causes a slough of the mucosa "

In 1947 Sanders constructed a high volume slip-on cuff for use on a silver tracheostomy cannula, and Safar et al (1962) noted that this cuff could be used for up to four months during artificial ventilation without any major complications, but they were unaware of the reasons for these good results

The recommendation of Grimm & Knight was otherwise not heeded widely, and the majority of cuffs used throughout the world were of the low volume type The really great breakthrough for the use of cuffed tubes, however, came during the polio epidemics in Europe in the beginning of the 1950s and in the U.S A in the early 1960s, in artificial respiration in patients with respiratory insufficiency At about the same time intensive care began its rapid development and prolonged mechanical ventilation of severely injured patients became increasingly common Previously the lives of such patients often could not be saved, so that

* Technical terms used in this thesis are explained in the chapters "Definitions" and "Cuff characteristics" (p 11 13)

complications of the artificial ventilation in itself never came to light. Now, when an increasing number of patients could be cured, the wider use of low volume cuffs gave rise to a considerable increase in reports of ulceration on the tracheal wall, tracheomalacias with consequent stenosis, and sometimes tracheo-esophageal fistulas This began to comprise a great problem all over the world, from the beginning of the 1960s onwards Clinically significant tracheal stenoses were reported in frequencies of up to 20% (Grillo 1970)

In view of the constantly increasing frequency of complications, during the second half of the 1960s analyses of the cause of the tracheal injuries were initiated in several centers

Some of the more important investigations concerning tracheal damage caused by tracheal cuffs were undertaken in Scandinavia by Lomholt (1967) and Lindholm (1969)

In the U S A and Canada this problem was studied by research groups in Pittsburgh (Carroll et al 1969, Hedden et al 1969), Boston (Cooper & Grillo 1969, Geffin & Pontoppidan 1969) and Toronto (Bryce et al 1968)

The general conclusion from these investigations was that the small resting diameter, small residual volume cuff (hereinafter called the *small cuff*) has to be inflated with a pressure of sometimes up to 200 mmHg just to reach the tracheal wall (Carroll et al 1969) Owing to its low residual volume, the cuff is stretched—to varying degrees—when the tracheal wall is reached In this way the cuff wall itself will take up an unknown proportion of the pressure in the cuff, which means that an idea of the cuff to tracheal wall pressure (C T pressure) cannot be obtained by measuring the intracuff pressure In clinical practice uncontrollable and often very high C T pressures were considered to be the fundamental cause of the tracheal injuries following prolonged tracheal intubation Yet another cause of the frequent occurrence of high C T pressures with the use of small cuffs was found For every superfluous milliliter of air that was insufflated into the cuff "for safety s sake a steep rise of the C T pressure was noted (see slope pressure under "Definitions" and Cuff characteristics p 11 13)

These experiences led to the recommendation by several authors (Lomholt 1967, Carroll et al 1969, Cooper & Grillo 1969) that the small cuff be replaced by the large resting diameter, large residual volume cuff, in the following called the *large cuff*

Lomholt (1967) states "The only simple way to control the lateral pressure of the cuff is to produce a cuff having an inside pressure exactly equal to the pressure on the tracheal wall These requirements are fulfilled if the cuff is made of a thin inelastic plast film and so large that it will never be fully expanded, but will lie in folds against the tracheal wall ' Lomholt proposed a cuff diameter of 35 mm

On the basis of simultaneous measurements of the intracuff pressure, airway pressure and C T pressure in anesthetized dogs, Carroll et al (1969) claimed that with *any* large cuff the intracuff and C T pressures are identical (as long as there is no circumferential tension in the cuff wall) that there is a low slope pressure at inflation beyond "sealing", and that sealing is achieved at low C T pressures, all compared with small cuffs They also found that the intracuff pressure automatically cycles in synchrony with the airway pressure without use of any gadgets so long as the cuff is of adequately large diameter and large volume

This means that the resting C T pressure with large cuffs can be much lower than the peak airway pressure and yet provide a seal Thus the *mean pressure* against the tracheal mucosa can be much lower than the peak tracheal pressure That is, if the patient is being ventilated at a pressure of 30 centimeters of water, and if an intracuff pressure of 15 centimeters of water is found adequate to prevent aspiration then the patient s tracheal mucosa will be exposed to a pressure of 15 centimeters of water during the two thirds of the time that he is in passive exhalation up to a pressure of 30 centimeters of water at the end of the one third of the time that he is undergoing positive pressure lung inflation

To compensate for the scarcity of acceptable commercially available large cuffs Geffin & Pontoppidan (1969) prestretched small plastic cuffs and converted them into large ones Those who

followed this recommendation noted a clear decrease of acute tracheal injuries after intubation (Grillo et al 1971)

Large cuffs of good quality became available, however, during the first half of the 1970s, and the use of this type of cuff has since increased. Tracheal surgeons all over the world have noticed a considerable reduction of the frequency of clinically significant cuff induced tracheal stenosis

In recent years several convincing investigations have demonstrated a reduction of cuff induced tracheal injuries with the use of large cuffs as compared with the small type (Grillo et al. 1971, Ching et al 1971, Dunn et al 1974, Mathias & Wedley 1974, Paegle & Bernhard 1975)

In the present investigation interest was therefore focused on the *large cuff* and no comparative studies were made between the large and small types. The hitherto most extensive investigation of the physical properties and functional aspects of the large cuff is that published by Carroll et al (1974)

Several different types of *safety devices* have been tried over the years with the aim of diminishing tracheal damage by the cuff

The pilot balloon used by Eisenmenger (1893) and the holed tube described by Wilms (1904) and Mushin (1943) have been mentioned above

Lauria & Andersen (1969) connected the cuff inflation tube to a side arm at the ventilator connection to the tracheal tube. This prevented the connecting holes from being plugged with secretion. Neither this nor Wilm s system however, gave full protection against aspiration in the expiratory phase

Lomholt (1971) desribed a system in which the cuff is inflated from a separate low pressure gas source during expiration. This equipment is not yet commercially available

Kamen & Wilkinson (1971) suggested a different solution to the problem. Their device consists of a large polyurethane foam filled cuff from which the air is aspirated at intubation. The insufflation channel is then left open whereupon the foam cuff expands by its own force which however, is adjusted so that a high C T pressure cannot occur *if the relation between the cuff and tracheal diameters is optimal*. This cuff seems to function well, although a problem may arise in that the foam may change shape with time, possibly related to the absorption of moisture, allowing audible air leak to occur at 18 to 36 hours of intubation. The cuff is available on the market but is expensive

McGinnis et al (1971) has connected the cuff to a pressure limiting balloon which maximates the intracuff pressure to 18 mmHg even when the cuff is inflated with up to 36 ml more air than is required for no leak ventilation. The system is commercially available and seems to work well. However, if the patient happens to lie on the balloon, or if this is compressed inadvertently by the personnel, the intracuff pressure will rise

Specific background of this study

Several decades ago, in 1943, Grimm & Knight pointed out the risk of *local ischemia* of the tracheal mucosa due to excessively high pressure from the cuff wall. In a large number of subsequent investigations in this field it has been assumed that local ischemia is a very important cause of tracheal injuries following endotracheal intubation. In practically every study on this subject speculations have been made on the level of C T pressure at which the ischemia might occur. To analyse this matter further, it was considered of interest to ascertain the perfusion pressure in the rabbit tracheal mucosal capillaries, in order to obtain an idea about when the pressure of a tracheal tube cuff on the mucosa can be expected to stop the capillary blood flow, i e to cause local ischemia, with a consequent risk of tracheal damage and stenosis

The aim of the present investigation was to elucidate the effect of intubation with a cuffed tracheal tube on the rabbit tracheal wall. Interest was focused on the importance of the shape construction and handling of the inflatable cuff

An attempt was made to answer the following specific questions

1 What can be done to minimize tracheal damage at intubation?
2 Does a safe C T pressure exist?
3 If so, what pressure range may be generally regarded as safe?

The problems were approached with respect to

1. Ultrastructural aspects (Papers I and II), *phase contrast microscopy* and *transmission and scanning electron microscopy were used to* study the mucosal surface damage caused by the cuff
2 The vascular anatomy of the tracheal wall (Paper III), here the normal anatomy was studied and compression of the mucosal vascular bed by an inflated large cuff was demonstrated
3 Functional aspects of the tracheal mucosa (Papers IV and V), this part of the investigation concerned blood flow, both under normal con ditions and under the influence of an inflated cuff, and the fluid balance

Definitions

Airway pressure The pressure within the trachea Airway pressure is further described by the terms end inspiratory, end expiratory, peak, and mean Airway pressure caused by positive pressure lung inflation often influences tracheal wall pressure exerted by a tracheal cuff

Aspiration protection Prevention of contamination of the lower airway with liquid or solid particles This does not necessarily imply prevention of bacterial contamination

Cuff diameter The outer diameter of a cuff which has been inflated at a pressure of 15 cm H_2O (11 1 mmHg) and allowed to deflate passively to a pressure of 1 0 cm H_2O (0 73 mmHg)

Cuff to tracheal wall pressure That force per unit area (pressure) which is exerted upon the internal tracheal wall by the cuff at various sites Cuff to tracheal wall pressure (C T pressure) is further described by the terms end inspiratory, end expiratory peak and mean C T pressure *SHALL* be measured at the anterior tracheal wall

High volume cuff Synonymous to large cuff

Inflating tube The tube provided for inflating and deflating the tracheal tube cuff

Large cuff See cuff characteristics (p 13)

Large resting diameter cuff Synonymous to large cuff

Large residual volume cuff Synonymous to large cuff

Low volume cuff Synonymous to small cuff

No-leak ventilation The condition in which a pre set measured volume of gas is delivered to test lungs, or the lungs of a subject, and the same volume of gas is exhaled through the lumen of the tracheal tube. No gas leakage between the inflated cuff and the trachea shall occur during no leak ventilation

Pilot balloon A balloon which may be fitted to the inflating tube to indicate inflation of the cuff

Prestretch A small plastic cuff is heated in boiling water The cuff is overinflated, thereby distending the cuff wall in such a way that the small cuff is converted to a large cuff The cuff is allowed to cool while still inflated

Prolonged use Any cuff or tube intended for use in a patient's trachea for a continuous period of about twenty four hours or more is considered a device for prolonged use

Residual volume The volume of air which can be withdrawn from the cuff after it has been inflated at a pressure of 15 cm H_2O (11 1 mmHg) and allowed to deflate passively to a pressure of 1 0 cm H_2O (0 73 mmHg) and assume its natural shape in the air This volume is measured at atmospheric pressure

Resting diameter See cuff diameter

Shearing force In this investigation this term is used to express the force causing mucosal damage by motion of the trachea relative to the cuff

Slope pressure That rise in pressure against a non rigid simulated human tracheal wall which occurs when 1 ml of air is added to a cuff which has been inflated to the minimum pressure

1 What can be done to minimize tracheal damage at intubation?
2 Does a safe C T pressure exist?
3 If so, what pressure range may be generally regarded as safe?

The problems were approached with respect to

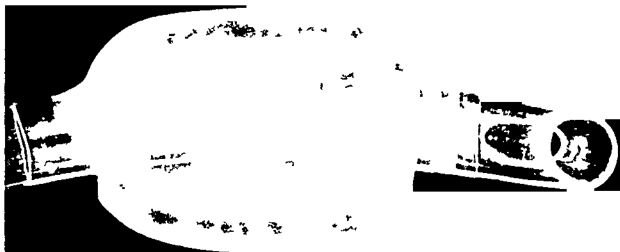
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Cuff characteristics

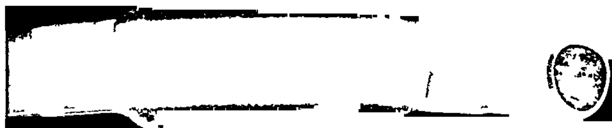
	Large cuff	*Small cuff*
Cuff diameter	Shall well exceed tracheal diameter	Less than tracheal diameter
Residual volume	More than 30 mL	3 – 6 mL
Cuff wall	Thin, pliable	Thick, rigid
Cuff pressure in relation C T pressure	Equal	Different Varying difference
Cuff pressure when in flated so as to just reach tracheal wall	Close to zero	High (75 –200 mmHg)
C T pressure needed for no-leak	Low 5 20 mmHg	High and uncontrollable
Cuff wall behaviour when cuff inflated to no-leak	Not distended Drapes freely over the tra cheal wall Lies in folds	Distended Often expands asymmetrically
Cuff pressure in rela tion to airway pressure	Equal at any moment that airway pressure exceeds intracuff pressure Airway pressure is able to reduce the cuff volume by squeez ing the pliable cuff wall facing the trachea	No relation Cuff wall too rigid and in-tracuff pressure too high
Area of cuff in contact with trachea at no-leak	Large	Small
Slope pressure	Low	Steep rise

Summary

A large resting diameter in relation to the size of the trachea is a fundamental prerequisite for the ability of a cuff to seal in such a way that the in tracuff pressure equals the C T pressure As soon as there is circumferential tension in the cuff wall, the C T pressure no longer equals the intracuff pressure









a Large cuff
b Small cuff

Material and methods

Rabbits were used for the experiments Dogs would have been preferable in regard to size, but for cost reasons this was not possible

Mostly white rabbits (New Zealand white strain) were employed, but seven grey rabbits (Dutch ram) were also used The animals were of both sexes and weighed from 0 9–4 4 kg, the ma jority being about 2 kg

The rabbits were anesthetized either with urethane 20 % in saline, in a dose of 1 g/kg body weight, or with 2 % sodium pentobarbital (Nem butal[R]) in a dose of 50–60 mg/kg body weight The anesthetics were administered by intravenous injection through an ear vein The animals breathed spontaneously throughout the ex periments

Intubation, tube and cuff

Translaryngeal *intubation* was performed under direct observation with the aid of a specially made laryngoscope Great care was taken to minimize tracheal damage from the intubation itself At in tubation the cuff was completely deflated in order to diminish shearing forces

The intubation *tube* was made of PVC and was specially constructed (by Portex) for this project The tube had an inner diameter (ID) of 2 5 mm and an outer diameter (OD) of 3 5 mm

The *cuff* was prestretched in hot water by the author (cf Geffin & Pontoppidan 1969), giving it large cuff properties (Lomholt 1967, Carroll & Grenvik 1973, Carroll et al 1974) in relation to the rabbit trachea After prestretching, the resting diameter of the cuff was about 10 mm The thickness of the cuff wall varied between 0 07 and 0 12 mm

In practice the cuff might fold and form six layers of cuff wall between the tube and the inner wall of the trachea An extreme total OD, the deflated cuff included, could thus have reached 3 5 + (6 x 0 12) = 4 22 mm in these experiments In addition, the elasticity of the cuff wall might have added a little to this extreme OD, so that in un favourable circumstances it would have ap- proached the ID of the trachea, which was about 5 mm This should be borne in mind when inter preting the results Radiography of the cuff site, however, showed a clear air gap between the tube and the tracheal wall, indicating that there was usually enough space in the trachea to accom modate the deflated cuff without undesired pressure on the tracheal wall

The cuff pressure was continuously monitored via a Y connector, with a mercury manometer, ac cording to Cox & Schatz (1974)

Paper I

Surface structure and vascular anatomy of the tracheal wall under normal conditions and after intubation

U Nordin, B Engstrom, B Jansson and C -E Lindholm

White rabbits were used to study the structure of the normal tracheal wall and to analyse the effect of a large tracheal tube cuff on the mucosa The specimens were examined by direct observation, using a binocular preparation microscope (Wild M 5), and by phase contrast microscopy and scanning (SEM) and transmission (TEM) electron microscopy

The *normal tracheal wall* was found to consist of a mucus membrane and a series of horse shoe shaped cartilages of the same appearance as in the human trachea. The surface layer of the mucosa is built up of a *pseudostratified columnar ciliated epithelium* containing many goblet cells This rests on a rather thick (1000 Å) basement membane, beneath which there is a loose network of connective tissue cells and fibers forming the lamina propria In this layer there is a rich capillary network The free upper surface of the cells is provided with large numbers of cilia

Between the cilia there are numerous microvilli The cilia are 6–7μm long and approximately 0 2 μm in diameter

The goblet cells or mucus producing cells are quite different in appearance from the ciliated cells, being goblet shaped with an upper widened portion and a slender foot These cells form secretory granules which fill the upper thecal portion of the cell *The free surface of the cells is often* richly furnished with microvilli

In this investigation the intubation time was kept constant at 15 min, while the C T pressure was varied from an uninflated cuff to a pressure of 100 mmHg

When the tube was placed in the trachea *without inflation of the cuff* it was quite clear that this was sufficient to cause minor superficial damage to the epithelial lamina This damage only occurred over regions where a cartilage was situated In the intercartilaginous regions no damage was seen

When the cuff was inflated, it resulted in an increase of the mucosal damage, the extent of the injury being directly related to the pressure in the cuff A gradual increase in C T pressure thus led to progressive extension of the mucosal damage This took the form of both widening of the injured areas and penetration of the damage to deeper regions In the damaged regions there was extensive cell loss, the epithelium was sometimes almost completely absent and only scattered cells remained

At a *C T pressure of 100 mmHg* the damage afflicted almost the entire mucosa and only small undamaged mucosal regions remained At this stage it appeared as if the basement membrane had also begun to disintegrate

Paper II

Surface structure of the tracheal wall after different durations of intubation

U Nordin, B Engstrom and C -E Lindholm

White rabbits were used and the specimens were studied by the same methods as in paper I

The aim of this investigation was to study tracheal damage caused by different durations of intubation, keeping the C T pressure constant. By study I and the present investigation an attempt was made to answer the question of which is the most important factor in causing mucosal damage, the C T pressure or the duration of intubation Another aim was to create a model by which "standardized" tracheal damage of different degrees could be produced to allow a study of the regeneration of mucosa injured to a fairly well controlled depth This study is in progress at present

The time of intubation was varied between one and four hours The results are presented in three different groups of C T pressures, with variations of the intubation time within each group The C T pressure in the first group was fairly 'normal , 20 mmHg, in the second group 'medium", 50 mmHg and in the third group 'high", 100 mmHg

A *C T pressure of 20 mmHg* for *1 to 2 h* of intubation damaged the mucosa on top of the cartilages to such an extent that it was partly denuded almost down to the basement membrane In intercartilaginous regions some of the cells had lost their cilia but the interior of the cells seemed intact The damage was not significantly greater than that after 15 min of intubation at the same pressure At *3 h* of intubation the damage was more widespread and in the intercartilaginous areas the epithelium was partly compressed

A *C T pressure of 50 mmHg* for *2 h* of intubation destroyed most of the epithelial cells on top of the cartilages The basement membrane was partly denuded but seemed intact Again, the damage was not significantly greater than after 15 min of intubation with the same C T pressure At *4 h* of intubation some parts of the mucosa in the intercartilaginous regions consisted of low epithelial cells covered by microvilli These cells were intermingled with normally ciliated cells Whether this was an expression of incipient disintegration of the cilia or a sign of incipient cilial regeneration, only 4 h after the damage was made, cannot be answered at present This question is being further elucidated in an investigation in progress at present (Nordin), where the regeneration of cuff induced mucosal damages is being studied

At a *C T pressure of 100 mmHg* for *1 h* of intubation the whole part of the mucosa at cuff level was now damaged to some extent Large areas of the basement membrane were denuded and in some areas it was completely absent, leaving the mucosal stroma visible After *4 h* of intubation the mucosa covering the cartilages was sometimes destroyed down to the cartilage itself, and bacteria were found, for the first time, to be invading the damaged mucosa This was the only time in all our experiments at which bacteria were observed It thus seems that even with fairly penetrating damage, local infection does not begin to play a serious role until after about 4 h of intubation

These investigations support the opinion of Mathias & Wedley (1974) that for "ordinary" periods of intubation, i e some hours, the C T pressure is more important than the duration of intubation for the occurrence of tracheal damage We are also convinced that in general the C T pressure has greater *relative* importance for the degree of damage than the intubation time

Paper III

The vessels of the rabbit trachea and ischemia caused by cuff pressure

U Nordin and C-E Lindholm

In order to consider the possibility of using the rabbit as a test animal for studies of the capillary perfusion of the tracheal wall under physiological conditions and during translaryngeal intubation, and to visualize the effect of an inflated tracheal tube cuff on the vascular bed, we injected Microfil silicone rubber* (Compound MV 118 plus diluent and curing agent) intraarterially When injected, the silicone rubber mixture is a fluid with a viscosity in the 15–25 centipoise range If the curing agent is added immediately before injection, however, the silicone rubber is transformed in 15 minutes into an elastomeric gel, which fills the vessels and thus preserves their anatomy

The rabbit was found to have large bilateral longitudinal arteries located outside the trachea and supplying the trachea and the esophagus We have named them Aa tracheo oesophageales longitudinales (the longitudinal tracheo esophageal arteries) (Nordin) They have connections with the bronchial arteries in the mediastinum and are mainly supplied by a branch of the right subclavian artery *The main branch* supplying the right longitudinal tracheo esophageal artery also has wide anastomoses with the intercostal arteries The main branch feeding the left longitudinal tracheo esophageal artery crosses the trachea from right to left anteriorly The longitudinal tracheo-esophageal arteries pass posterolaterally to the trachea in the groove between the trachea and the esophagus, giving off numerous segmental branches to both these organs The longitudinal tracheo esophageal arteries in the upper cervical region are supplied mainly by branches of the inferior thyroid arteries originating in the common carotid *arteries* All longitudinally orientated vessels are interconnected and give off arcades of semicircular arteries, which pass submucosally between the tracheal cartilages These intercartilaginous arcades are also connected with each other through the capillary bed of the mucosa covering the tracheal cartilages and through scattered small caliber arterial shunts between one semicircular artery and an adjacent one

In addition to the described vascular system, located mainly in the submucosa, there is a similar distribution of vessels close to the external surface of the trachea

Our studies also disclosed a system of sinusoidal ectasias, or cavernous vascular spaces, as suggested by Sobin et al (1963) These sinusoids, which are mainly located submucosally, lie in contact with small-caliber thin walled vessels of the tracheal wall They are lined with epithelium It is probable that they play a role in the conditioning of inhaled air

Translaryngeal intubation with a cuffed PVC tube was performed The cuff had been sufficiently prestretched to obtain large cuff characteristics in relation to the rabbit's trachea C T pressures of 50 mmHg always hindered the silicone rubber perfusion of the capillary bed in the tracheal mucosa in contact with the cuff

The conclusion drawn from this study was that the rabbit trachea has a rich vascular supply The small arteries form a network of anastomoses in all three dimensions within the tracheal wall The complexity of the arteriolar and capillary bed in the tracheal wall suggests that the rabbit trachea has a vascular bed very well prepared to resist a threat to its nutritive blood supply This fulfils an important prerequisite for the use of the rabbit as a test animal for quantitative studies of the effect of an inflated cuff on the capillary perfusion of the tracheal wall

* Canton Bio-Medical Products Inc P O Box 2017 Boulder Colorado 80302 U S A

Paper IV

Transvascular fluid exchange in the tracheal mucosa

U Nordin, O Källskog, C E Lindholm and M Wolgast

This study was undertaken because it was considered of interest to ascertain the perfusion pressure in the rabbit tracheal mucosal capillaries, to obtain an idea about when the pressure of a tracheal tube cuff on the mucosa can be expected to stop the capillary blood flow, i e to cause local ischemia, with a consequent risk of tracheal damage and stenosis

This study was also undertaken to evaluate the fluid balance in the tracheal mucosa, and is closely related to study V, which deals with the blood flow characteristics under normal conditions and after the insertion of the foreign body, (e g a tracheal tube) into the trachea It was clearly evident from the latter investigation that this will cause an irritation of the mucosa, resulting in a release of a vasodilating agent which with the greatest probability exerts its effect on the small arteries and arterioles in the tracheal microvasculature This was reflected by an increased blood flow Such dilatation will also mean an increased pressure in the capillary bed and it seems likely that this will enhance the outward filtration of fluid across the capillary wall into the mucosal interstitium primarily in the proximal part of the capillaries The generally increased pressure will also diminish the rate of reabsorption in the late segment of the capillaries and small veins in the submucosal region

In order to perform the micropuncture experiments (see below) it proved essential to eliminate movements of the tissue that normally occur e g movement of the trachea in the craniocaudal direction synchronous with breathing In this series of studies the mucosa was exposed via a midline incision in the ventral part of the trachea Two clamps were then attached to the edges and fixed to the operating table on both sides With this technique the tissue could be immobilized to a sufficient degree in most cases

In parallel with the micropuncture experiments the blood flow was analysed by the microsphere method (see below) Thus two series of experiments were carried out, comprising 1) studies of the mucosal blood flow and 2) micropuncture investigations The trachea was prepared and fixed in the same way for both series

Series I Blood flow investigations

The microsphere technique was used for determining the capillary blood flow in the tracheal mucosa The microspheres (see below) were administered through a catheter placed in the left ventricle A catheter in the left femoral artery was connected to a suction pump, which sampled at a constant rate of 0 82 ml/min The microspheres (141 Ce labelled and 85 Sr labelled 3M microspheres, St Paul, Minn , USA), numbering about 1 million, were suspended in 0 6 ml of rabbit plasma. For each determination of mucosal blood flow 0 6 ml of the plasma microsphere suspension was injected through the catheter into the left ventricle During the injection, and up to 1 min afterwards, blood was drawn continuously from the left femoral artery at a constant rate of 0 82 ml/min

It may be assumed that the injection of the microsphere plasma suspension into the left ventricle will ensure complete mixing of the microspheres The microspheres, which are 15 μm in diameter and homogeneously suspended in the

blood, will be carried by the blood to the different organs where they will be trapped in the capillary bed The number of spheres trapped in different tissues will be directly proportional to the blood perfusion of that particular tissue during the time of injection The first injection, as a rule with the 141 Ce labelled microspheres, was given under resting conditions, i e when the trachea was still intact The second injection, with 85 Sr labelled microspheres, was given after the trachea had been exposed and fixed with the clamp arrangement described above Pieces of the trachea in the region accessible for micropuncture, as well as from the region cranial and caudal to this site, were then freed by blunt dissection from the underlying perichondrium at the site of the tracheal cartilages, and from the annular ligaments between the cartilages, with the aid of an operation microscope With this mode of dissection the tissue pieces contained the mucosa in which, in accordance with the Nomina Anatomica, we include the tela submucosa The radioactivities of the tissue pieces and blood sample were determined

For calculating blood flow the formula $F/M = F_s/M_s$ was used, where F is the blood flow to the region in question, M the activity of the isotope, F_s the blood sampling rate of 0 82 ml/min and M_s the radioactivity in the blood sample Cardiac output was determined from the same formula, replacing the factor M by the total amount of radioactivity injected, the factor F is now the cardiac output

The mucosal blood flow in the region used for the micropuncture experiments was estimated at 0.62 ± 0.41 ml/min g tissue in the resting state Essentially the same values were obtained for the areas cranial and caudal to this region After the manipulation of the trachea the blood flow increased markedly to 3.22 ± 1.55 ml/min g and a similar increase was noted for the areas cranial and caudal to the region used for micropuncture It is thus clear that the manipulations of the trachea induced an arteriolar dilatation in the same way as on introduction of a foreign body The figures obtained in the micropuncture experiments will thus not apply to normal conditions but to the conditions of an irritated mucosa

Series 2 Micropuncture investigations

The experiments with the micropuncture technique aimed at exploring the microanatomy of the tracheal mucosal vascular bed and interstitium, and also at measuring the hydrostatic and colloid osmotic pressures and the fluid turnover Injection of dyes allowed identification of the early and late part of the capillaries as well as the venules It was found that the capillary network was oriented longitudinally in the layer below the tracheal epithelium The network was freely anastomosing and supplied with several arterioles It was drained by typical interconnecting vessels, which formed a loop from the superficially located true capillaries to the dense network of sinusoidal veins in the submucosal region These "venules" increased in size as they passed down to the submucosal veins The most prominent feature of the mucosal vasculature was the very dense net of sinusoidal veins in the submucosal region, which again was freely anastomosing Somewhat larger veins draining the network could also be identified The region between the capillaries and the layer beyond was occupied to a surprisingly large extent by a very richly developed lymphatic system The lymphatic tree ended in sac like structures which were designated terminal lymphatic sacs The capillaries, venules and submucosal veins were all permeable to the dye Thus when the dye was injected the greatest part followed the blood stream but some of it diffused out into the interstitium and then returned to the blood stream This process was complete within a few seconds Concerning the lymphatic system, the connecting lymph ducts proved to be fairly impermeable to the dye, the only site of passage being the above mentioned terminal lymphatic sacs The dye injected into the interstitium was first collected by the walls of the lymphatic sacs and then passed into the collecting lymphatic channels, this process was fairly slow, with a turnover time of about ½ hour

The hydrostatic pressures within the microvasculature were investigated by means of sharpened glass capillaries with a tip diameter of 2-4 μm These were attached to the servo nulling device of Wiederhielm (1964) in a modification by Intaglietta et al (1970) The early part of the

capillaries was denoted as the first branch distal to an arterial branch, the latter being seen as a red dot which split up into a capillary network The "mid-capillary" pressure is in fact the mean pressure in randomly punctured capillaries that were not adjacent to arterioles or venules The late part of the capillary is easily identified as the part in the immediate vicinity of the venules described above. The capillary pressure decreased from 28 mmHg in the early part of the capillary to 17 mmHg in the mid part and about 14 mmHg in the late part. The pressure in the sinusoidal submucosal veins was 12 mmHg The pressure in the interstitium and terminal lymphatics was 3–4 mmHg

The colloid osmotic pressure in plasma was determined from the protein concentration in systemic blood and was found to be 6 1 ± 0 2 % The protein concentration in the interstitial fluid or lymph fluid was 5 7 ± 0 8 %, as obtained from small samples withdrawn by sharpened glass capillaries with an outer tip diameter of about 10 μm The sample volume was fairly high—about 50 nl or more The colloid osmotic pressures would then be 21 mmHg and 19 mmHg in plasma and interstitial fluid, respectively At these values there would be an outward filtration of fluid from all the vascular structures, i e both from the capillaries and from the sinusoidal submucosal veins The driving force was 22 mmHg in the early part of the capillary, decreasing to 6 mmHg in the venous plexus The most remarkable finding in this context was the high colloid osmotic pressure, of 19 mmHg, in the interstitium This will reflect a heavy leakage of proteins through the capillary membrane The transfer sites could be either large pores or a pinocytotic process, the present experimental set up will not allow a more detailed evaluation of the transfer route

The transport of plasma proteins was investigated in an additional study in which labelled albumin was injected into the systemic circulation and a series of interstitial fluid samples was obtained from time zero up to 2 h after the injection A transit time of about 45 min was noted The short time of 45 min will thus reflect a very high rate of transport of the proteins. It is thus concluded that the fluid entering the interstitium will be composed of a protein free filtrate, most probably passing through a small pore system In addition there will be a comparatively high outflow of proteins The final concentration of proteins in the filtrate will not necessarily equal that in the interstitium

From the above results it is suggested that the interstitial fluid is drained by two separate routes Part of the fluid will be reabsorbed by the secretory glands for the production of mucus This part is essentially a protein free fraction, as the protein concentration in the mucus is essentially nil Due to this process the protein concentration in the interstitial fluid will increase, which is probably the explanation for the very high concentration of proteins in the interstitium of 5 7 % The drainage of this part of the fluid will occur through the terminal lymphatic sacs into the collecting lymphatics

The process described seems to suit the demands on the tracheal mucosa of an increased production of mucus in the case of introduction of a foreign body Thus the foreign body will cause an irritation of the mucosa, resulting in release of a histamine like substance or an equivalent vasodilator agent. The dilatation of small arteries and arterioles will obviously lead to an increased blood flow but also to increased pressures in the mucosal microvasculature. The high blood flow and the high pressures will favor the outward filtration of fluid to supply the secretory glands with increased amounts of fluid to be secreted The high rate of mucus production will assist the trachea in its attempt to remove a foreign body which has originally caused this reaction The question then arises if there is any need for a higher rate of protein transport. From the point of view of the secretory glands plasma proteins will be unnecessary, for the simple reason that mucus is essentially protein free It could, however, make sense with respect to antibody proteins, which will now gain access to the mucosa. It has been shown, in fact, that in the case of allergic reactions there is an increase in the protein concentration in the mucus (though only trace amounts), especially the concentration of IgE (Deuschl & Johansson, 1977)

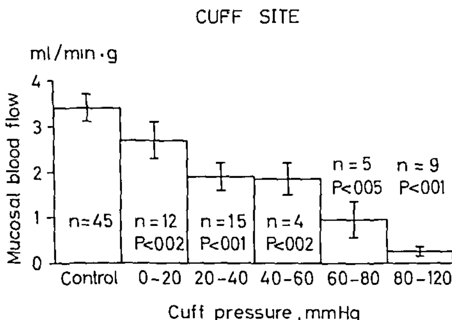


Fig 1 Mucosal blood flow under control conditions, i e with the tube inserted but with no inflation of the cuff, and the flow during different cuff pressures, which are grouped here in steps of 20 mmHg It is seen that the flow decreases with increasing cuff pressures, approaching zero at cuff pressures of 80—120 mmHg, i e at pressures corresponding to the systemic arterial pressure

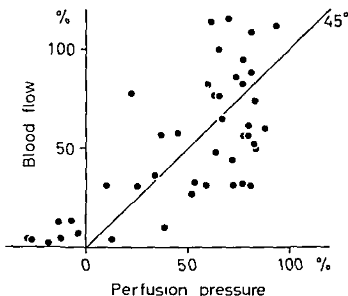


Fig 2 Relation between the tracheal mucosal blood flow, expressed here as a percentage of that under control condition (ordinate), and the "perfusion pressure" (abscissa), calculated as the difference between systemic arterial pressure and cuff pressure, and expressed as a percentage of that under control conditions (see text) Even when the calculated perfusion was zero or negative there was still some blood passing through the tracheal mucosa The calculated perfusion pressure was thus not identical with the true perfusion pressure The reason for this was probably that the large cuff used in these experiments did not have ideal properties The discrepancy from the ideal state was, however, quite small

plained as being due to errors in the estimation of the true perfusion pressure In the case of high cuff pressures it is evident that there was still some blood flow in the mucosa in spite of a zero or even negative perfusion pressure It is reasonable to explain this finding by assuming that in some places the cuff pressure was not evenly transmitted to the vascular structures, i e the pressure exerted by the cuff in some places was lower than the intracuff pressure This would then indicate that the large cuff used does not function as an "ideal" large cuff (see "General discussion", p 26, for details)

General discussion

It seems quite clear from this investigation that any intubation causes a certain amount of surface damage to the tracheal mucosa, no matter how short its duration, or how low the C T pressure This vulnerability of the mucosa was indicated from the early experiments of Hilding (1952), and our finding is in accordance with the observations of Klainer et al (1975)

An increase in C T pressure will lead to progressive extension of the mucosal damage The basement membrane comprises an important protective layer against deep ulceration Thus in the present studies it was not disintegrated until a C T pressure of about 100 mmHg was applied, if the time of intubation was kept at only 15 minutes When the same C T pressure was exerted for four hours, on the other hand, the mucosa covering the cartilages was sometimes destroyed down to the cartilage itself

It was obvious that the damage began and was most pronounced at the mucosa covering the tracheal cartilages This is explained by the fact that the wall of the cuff used was not sufficiently thin and pliable in relation to the smallness of the rabbit trachea This emphasizes the importance of the *quality of the cuff wall* The thicker and more rigid it is, the more will the cuff act as a small volume type, i e it will ride on the edges of the cartilages, where it will cause deep ulceration This will happen even if the resting diameter of the cuff is large enough to provide it with large cuff properties in relation to the trachea

It must also be underlined that the *large resting diameter* of the cuff is the crucial factor in allowing the cuff to seal in such a way that the intracuff pressure will equal the C T pressure It must be kept in mind, on the other hand, that the large resting diameter of the cuff will also allow high intracuff pressures to be transmitted undiminished to the tracheal wall until such time as the diameter *of the trachea exceeds the diameter of the cuff*, when *circumferential tension within the cuff* will start to limit the effective C T pressure At such a time, the trachea is already dilated, and the physics of seal is changed from that of the large cuff to that of the small (high pressure) cuff It is thus important to control the intracuff pressure and the resulting tracheal diameter

When the length of intubation was extended to two hours, the damage was not significantly greater than that seen after 15 minutes of intubation at the same C T pressure Our findings are thus in accordance with the report by Mathias & Wedley (1974) that for "ordinary" periods of intubation, i e some hours, the C T pressure is more important than the duration of intubation for the occurrence of tracheal damage We are also convinced that in general the C T pressure has greater *relative* importance for the degree of damage than the intubation time

When a foreign body, e g a tracheal tube, is inserted in the trachea, this causes a steep increase in the blood flow through the tracheal mucosa This increase would seem to have a dual purpose

1 It causes an enhanced outward filtration of fluid across the capillary wall into the mucosal interstitium Part of this fluid will be reabsorbed by the secretory glands for the production of mucus The *high rate of mucus production* will assist the trachea in its attempts to *remove* the foreign body which originally caused the reaction

The irritation of the mucosa will also cause a heavy leakage of proteins through the capillary membrane into the interstitium This will result in a raised colloid osmotic pressure, which will disturb the fluid balance, giving a great *risk of edema* in the tracheal mucosa

This risk will be *diminished* however, on changing the body posture from a recumbent to an *upright position* as this will lead to a decreased pressure within both the capillary and the venous system (Jonson & Rundcrantz, 1969)

2 When the organism reacts to the presence of a foreign body with local hyperemia, this probably is a way of meeting a possible threat to its nutritive blood supply This must play an important role in diminishing the effects of local mucosal ischemia caused by an inflated small tracheal tube cuff

On the basis of our findings the different effects of a small and an *ideal* large cuff on the mucosal blood perfusion may be analysed in a *theoretical model* of the trachea The *ideal* large cuff has very thin and pliable walls, which drape freely over the mucosa, allowing the C T pressure to be transmitted to the mucosa overlying the cartilages, as well as to the mucosa over the intercartilaginous spaces The pressure upon the mucosa trapped between cuff and cartilage may be somewhat higher than that on the intercartilaginous mucosa, where the force is dissipated in a relatively freely distensible tissue But the more ideal the cuff, the more this relative pressure difference will be diminished It is probable, further, that even on slight dilatation of the trachea, caused by the cuff, the annular ligaments

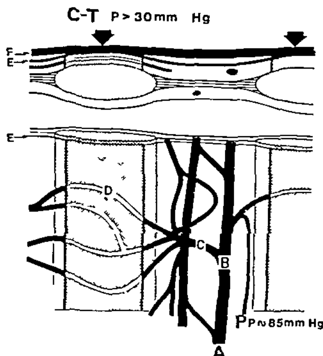


Fig 1 The effect of a small cuff on the blood perfusion Pressure is exerted mainly on the mucosa covering the cartilages (shaded) C T pressures (see text) exceeding 30 mmHg will impede the capillary perfusion in this region Pp perfusion pressure at B (F = mucosal epithelium F = cuff wall)

between the cartilages will start to back the mucosa, which will contribute to reducing the above mentioned pressure difference

Figure 1 shows a schematic drawing of the vascular anatomy of the trachea At A in the figure, an artery is passing between the tracheal cartilages, to form an arteriole at point B The arterioles then split up into a capillary network Some of these capillaries supply the mucosa between the cartilages, while others traverse the mucosa, covering the cartilages The mean systemic blood pressure in the experimental series was about 95 mmHg (point A)

At branching point B the pressure was estimated to be about 85 mmHg The arteriolar pressure, as measured by micropuncture techniques in study IV, was 30 mmHg and the mean pressure within the capillary network was 20 mmHg The micropuncture experiments also showed that blocking of a single capillary with a small glass rod led to a pressure increase in the capillary to 30 mmHg, which is equal to the pressure at the branching point (C) proximal to the site of puncture (D) Similarly, when the arteriole was blocked at C, the pressure proximal to the blocking point rose to about 85 mmHg, i e to the pressure at branching point B

When the information obtained in papers IV and V is applied to a *theoretical model* of the trachea, to examine the effects of a small cuff and an *ideal* large cuff the following circulatory events can be expected

In the upper part of Figure 1 the effect of a small cuff is illustrated schematically The cuff wall is thick and unpliable i e it exerts its pressure only on the mucosa covering the cartilages, leaving the intercartilaginous mucosa intact

When the pressure from the cuff exceeds 20 mmHg, the capillaries at D in Figure 1 are blocked This leads to an increase in the perfusion pressure to 30 mmHg, i e to the pressure at the proximal branching point C, and the blood perfusion is maintained When the pressure from the cuff exceeds 30 mmHg, and the capillaries are again blocked, there will be no further increase in the perfusion pressure, as the arteriole is not compressed by the cuff, being drained through the capillaries located between the cartilages and thus untouched by the cuff This means that when the pressure from the cuff exceeds 30 mmHg, local ischemia will occur in the mucosa covering the cartilages (shaded region in Fig 1) This is in accordance with our clinical observations that the small cuff causes ulceration of the mucosa covering the cartilages

In the upper part of Figure 2 the effect of an *ideal* large cuff is illustrated The cuff completely drapes the inside of the trachea and exerts almost the same pressure over the entire mucosa A C T pressure of 20 mmHg blocks the capillaries at D The perfusion pressure is raised to 30 mmHg, and the blood flow continues When the C T pressure exceeds 30 mmHg, the arteriole (C) is also blocked, since in this case the arteriole is not drained by the capillaries located between the cartilages, as these themselves are blocked by the cuff This means that with rising C T pressure, the perfusion pressure will also be increased and will be propagated further out into the capillary bed *Theoretically*, not until the C T pressure exceeds about 85 mmHg, i e the pressure at point B, will complete ischemia develop and this will involve the entire mucosa

Thus when the *ideal* large cuff exerts a C T pressure exceeding 20–30 mmHg, the blood flow perfusion in the mucosa will be considerably decreased, but on the other hand the nutritive blood flow will not completely stop until C T pressures far exceeding 30 mmHg are reached *Thus the tracheal vascular supply seems to have* an inbuilt mechanism which tends to preserve a certain blood perfusion, even in the mucosa covering the cartilages, when pressure is exerted by a large volume cuff at least for short periods of time

This adds to our understanding of why large cuffs seldom cause a clinically significant mucosal necrosis upon brief use of fairly high C T pressures It has been shown by Stanley et al (1974) and Stanley (1975) that nitrous oxide will diffuse into a cuff filled with air during nitrous oxide oxygen anesthesia and cause a pressure increase Further, Revenas & Lindholm (1976) reported that C T pressures far exceeding 30 mmHg are commonly found after some hours of this anesthesia but in spite of this clinically

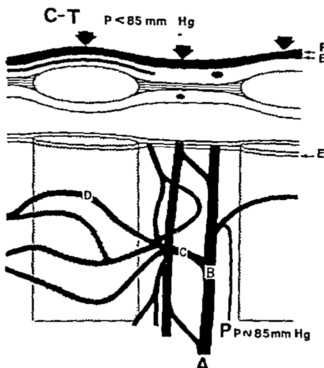


Fig 2 The effect of a large cuff on the blood perfusion Here pressure is exerted evenly over the entire mucosa draped by the cuff The blood perfusion will not cease completely until the C T pressure exceeds 85 mmHg (see text for details) Pp perfusion pressure at B (F – mucosal epithelium F = cuff wall)

significant tracheal wall damage has rarely been reported since the introduction of the large cuff

If during nitrous oxide oxygen anesthesia extremely high C T pressures (in the range of 50–100 mmHg) are exerted over a period of some hours it is only natural that the large cuff will then cause more sore throats than the small cuff as remarked by Loeser et al (1976) The thinner and the more pliable the cuff wall relative to the tracheal mucosa the greater the surface area upon which this very high sealing pressure is distributed which means that under this unfavourable condition the large cuff will damage a larger area of the trachea than a small cuff

However poor cuff pressure control is to be blamed for this damage rather than the large cuff itself If the large cuff is inflated with the same anesthetic gases as are used for the anesthesia the C T pressure will be kept essentially unchanged during the course of the anesthesia

Moreover measurement of the intracuff pressure (=C T pressure) at least when the cuff is being inflated, is the best guarantee for a safe cuff seal in the trachea.

The so called parachute cuff is a way of avoiding dangerous overinflation of the cuff that may occur either by a mistake on the part of the staff or by diffusion of nitrous oxide into the cuff during nitrous oxide-oxygen anesthesia This cuff was first described by Martinez (1964) and Jackson & Rokowski (1967) The first one that seems to have provided a good seal however is that described by Merav (1971)

The parachute cuff has holes in the part of its wall that faces the bronchial tree The airway pressure will be propagated through these holes and will thus inflate the cuff to exactly the pressure needed for sealing but no higher This means that the staff will not need to inflate the cuff through a special inflation tube Neither will the cuff

pressure need to be monitored As the cuff functions as an open system, there is no risk of an undesired pressure increase through diffusion of nitrous oxide into the cuff

The disadvantage of the parachute cuff is its inability to prevent aspiration when the airway pressure, at the end of an exhalation, is zero or even negative

In a preliminary report Nordin & Lyttkens (1976) described a modified parachute cuff which, if used with a respirator set at a positive end expiratory pressure (PEEP), both provided a seal and protected against aspiration This cuff has an increased length, extending up into the larynx, which prevents pooling of secretions above the cuff and will give a greater sealing area against the tracheal mucosa The cuff has now been tested further on dogs and a full report is under preparation (Nordin & Lyttkens) The cuff always provided a seal A PEEP of 7–10 cm H_2O gave full protection against aspiration, as tested by oral instillation of contrast medium under fluoroscopic control

If this cuff is applied outside a standard large cuff, aspiration can always be prevented If the PEEP disappears, e g on suction through the tube, the first measure is to suction the mouth clean The inner cuff is then inflated in the usual way and full protection against aspiration will be achieved During mechanical ventilation with PEEP, the inner cuff should be deflated, so that it does not block the holes in the walls of the outer cuff The modified parachute cuff is especially suitable for use with high frequency positive pressure ventilation

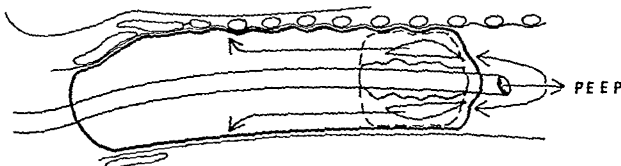


Fig 3 Modified parachute cuff

General conclusions

1 Any intubation causes a certain amount of surface damage to the tracheal mucosa

2 Tracheal cuff damage is highly influenced by the product of C T pressure and duration of intubation

3 Of these factors, C T pressure has the greatest importance

4 Any irritation of the tracheal mucosa or disturbance of the mucosal lymph drainage will lead to a great risk of mucosal edema, and also favour a higher rate of mucus production, which will assist the trachea in its attempt to remove a foreign body that has originally caused this reaction The risk of mucosal edema will be lower with the patient in an upright position

5 A small cuff will stop the microcirculation in the mucosa covering the tracheal cartilages at C T pressures as low as 30 mmHg

6 A large cuff with thick and rigid walls will tend to produce lesions similar to those caused by a small cuff

7 An *ideal* large cuff would considerably decrease the blood perfusion rate above C T pressures of 30 mmHg but on the other hand it would not completely stop the capillary blood perfusion of the tracheal mucosa until C T pressures far exceeding 30 mmHg were reached No cuff, however, has this property and we cannot therefore give general figures for a safe C T pressure range, as this will vary with the quality of the cuff

8 Although there are physiological mechanisms which preserve some microcirculation even at fairly high C T pressures when an *ideal* large cuff is used, the C T pressure should be kept below 20 mmHg Pressures above this level may be regarded as not being catastrophic for a limited time period but should absolutely not be abused Monitoring of the cuff pressure is therefore strongly recommended

9 The walls of the *ideal* large cuff should be as thin and flexible as possible, so that it will drape evenly over the mucosa The best guarantee for maintaining a satisfactory blood flow along the entire mucosa and for preventing a risk of local ischemia would be that the cuff wall should be so thin as to be almost non-existent, thus as nearly as possible giving the cuff the quality of an air cushion

10 *C T pressure below 30 mmHg is clearly associated with better blood flow and better histology than higher pressure*

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Surface structure and vascular anatomy of the tracheal wall under normal conditions and after intubation

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Abstract The effect of a large tracheal tube cuff on the rabbit tracheal mucosa was investigated by phase contrast microscopy and scanning (SEM) and transmission (TEM) electron microscopy The tube was left in the trachea for 15 min The cuff was either uninflated or inflated to a cuff to tracheal wall pressure (C T pressure) of up to 100 mmHg

The uninflated cuff caused superficial damage to the epithelial lamina over regions where a cartilage was situated

When the cuff was inflated it resulted in an increase of the mucosal damage the extent of which was directly related to the pressure in the cuff This took the form of both widening of the injured areas and penetration of the damage to deeper regions At a C T pressure of 100 mmHg the damage involved almost the entire mucosa and only small unaffected mucosal regions remained At this stage it appeared as if the basement membrane had also begun to disintegrate

It is well known that a *small* cuff easily causes deep ulceration in the mucosa overlying the cartilages

From this investigation it was concluded that a *large* cuff causes the same type of ulceration if 1) the cuff wall is not sufficiently thin and pliable and 2) if the cuff is overinflated enough to dilate the trachea to a diameter exceeding the cuff diameter At that moment there will be circumferential tension in the cuff and the sealing physics of the large cuff will become the sealing physics of a small (high pressure) cuff

A large cuff properly handled is more benign to the trachea than a small cuff In order to avoid overinflation of the large cuff the intracuff pressure (= C T pressure) should always be measured by means of a four way stopcock and an aneroid manometer In the case of extended periods of mechanical ventilation with a high airway pressure the resulting tracheal diameter at the cuff site should be checked radiographically

Introduction

Tracheal intubation is widely used in anesthesiology The rapid progress of intensive care during the last decade has made the use of cuffed tubes increasingly common In many cases the tube is left in situ for long periods Since many tubes and cuffs exert harmful pressure on the tracheal wall, prolonged intubation sometimes causes sufficient damage to result in a tracheal or laryngeal stenosis Consequently, the interest in the causative factors underlying tracheal stenosis has increased considerably all over the world Incidences of tracheal stenosis varying from 1 5 to 20 % have been reported (Pearson et al, 1968, Lindholm, 1969, Grillo, 1970) Investigations by Geffin & Pontoppidan (1969) indicate that the stenosis is most often found in the region where the cuff has exerted its main pressure against the tracheal mucosa The most widely used cuffs have been of the small resting diameter, small resting volume type, in the following called *small* cuffs Many publications have described the damage caused by this type of cuff and most authors have come to the conclusion that the main etiological factor is the high pressure exerted on the tracheal wall (Pearson et al, 1968 Cooper & Grillo, 1969, Shelly et al, 1969, Donnelly, 1969, Hedden et al, 1969, Grillo et al, 1971 Andrews & Pearson, 1971, Hilding, 1971, Magovern et al, 1972, Goldenberg & Pearson 1972)

It was against this background that many anesthesiologists strove to find better systems for tracheal "seals", and Safar et al (1962) established that large Sanders cuffs did not produce tracheal damage visible at tracheoscopy They had used these cuffs in humans over extended periods, but they were unaware of the reasons for these good results Clinical experience of a similar nature was reported by Lomholt (1967) Other authors (Carroll et al, 1969, Carroll, 1973) found in animal experiments that the design of the cuffs was of primary importance in determining the damage caused and that large resting diameter, large residual volume cuffs—called in the following *large* cuffs—were able to seal the trachea at a much lower cuff to tracheal wall pressure (C T pressure) than the small ones Similar findings were made by other authors (Geffin & Pontoppidan, 1969, Cooper & Grillo, 1969 and 1973, Ching et al, 1974)

The evidence from these pioneer investigations enhanced the interest in the design and construction of tubes and cuffs, and experience from human intubations (Ching et al, 1971 and 1974, Grillo et al, 1971, Mathias & Wedley, 1974, Paegle & Bernhard, 1975) and animal experiments (Cooper & Grillo, 1969, Magovern et al, 1972, Ching et al, 1974, Dunn et al, 1974, Klainer et al, 1975) has been reported by many authors

Some of these publications are of special interest with regard to the studies described in this paper Grillo et al (1969) reported on 8 dogs intubated from 5 to 14 days with large cuffs inflated to a constant pressure of 20—40 mmHg In one dog there was slight discoloration of the mucosa after 13 days, while the rest showed no macroscopic mucosal damage At light microscopy only mild inflammatory changes were seen

Magovern et al (1972) reported on dogs intubated for 3—30 days with a large cuff, where the cuff pressure was controlled and kept around 20 mmHg No macroscopic damage was seen After

30 days of intubation light microscopy revealed a well preserved mucosa with flattened epithelium They also reported on 3 patients in whom the cuff was inflated to 20 mmHg for 45 55 and 200 days respectively Endoscopic examination showed no evidence of mucosal damage

Dunn et al (1974) described observations on 8 dogs in which large cuffs were inflated to seal (0–10 mmHg) for 10 days After 5 days 6 of the dogs were subjected to a 12 h period of hypotension (systolic blood pressure 40–60 mm Hg) Three other dogs were on positive pressure ventilation for 96 h In 10 of these 11 dogs only microscopic hemorrhage was observed at the cuff site, with no mucosal damage In one dog there were gross mucosal hemorrhages and microscopic erosions at the cuff site The period of hypotension did not influence the extent of tracheal damage and this is in accordance with the findings of Shelly et al (1969) and of Mathias & Wedley (1974)

Paegle & Bernhard (1975) reported on 12 patients intubated from 2 h to 20 days with a large cuffed tube where the cuff pressure was kept between 20 and 25 mmHg. The cuffs usually did not even erode the entire thickness of the tracheal epithelium After more than a week of intubation preserved ciliated cells could be found in the inter cartilaginous regions

Klainer et al (1975) reported on scanning electron microscopic studies of cuff induced damage They intubated dogs with large cuffs for a period of 2 h In dogs in which the cuff was left uninflated transverse areas with loss of cilia were seen At a C T pressure of 18–25 mmHg they also found a diffuse area of ciliary denudation at the cuff site Light microscopy of the same areas revealed almost complete destruction of the mucosa however

When we started the present investigation all previous studies in animals and clinical investigations had employed tracheoscopy and histopathologic examination with light microscopy for evaluation of the damage

Tracheal damage caused by intubation continued, however, to be a problem needing further investigation. With the development of improved methods for structural research it was therefore considered of value to apply such techniques as improved light microscopy and scanning and transmission electron microscopy

Several convincing reports have already demonstrated a reduction in cuff site tracheal injury with large cuffs, as compared with small ones (Cooper & Grillo, 1969, Grillo et al , 1971, Ching et al , 1971, Dunn et al , 1974, Mathias & Wedley, 1974 Paegle & Bernhard, 1975) In the present study, therefore, we focused our interest on the effect of the *large* cuff, rather than on comparison with that of the small cuff

The aim of this investigation was to study

1) the normal tracheal mucosa,

2) the damage to the tracheal mucosa caused by intubation for a constant length of time with a tube equipped with a large cuff, inflated to different C T pressures

3) the vascular anatomy of the tracheal mucosa by arterial infusion of silicone rubber and the effect of an inflated large cuff on the vascular bed (For further details see Nordin & Lindholm, 1977 a)

In parallel we have studied

1) the effect on the mucosa of different durations of intubation keeping the C T pressure constant (Nordin et al., 1977 b),

2) the capillary blood perfusion pressure and fluid dynamics of the tracheal mucosa by the micropuncture technique (Nordin et al , 1977 c),

3) the effect of different C T pressures on the capillary blood flow of the tracheal mucosa (Nordin et al 1977 d)

Material and methods

Fifteen white rabbits, weighing 2–2 5 kg, were used Three of them were used for a study of the normal morphology of the mucosa, 2 for a study of the normal vascular bed and 10 for a study of the effect of intubation To ascertain whether the rabbit was as suitable as the cat for studies of the tracheal blood perfusion, one adult cat was used to visualize the vascular bed

The structure of the tracheal wall was examined by direct observation—using a binocular preparation microscope (Wild M 5), and by phase contrast microscopy and by scanning (SEM) and transmission (TEM) electron microscopy The techniques for SEM and TEM were those currently used in our clinic (Ades & Engstrom, 1972) For SEM the specimens were fixed in buffered 2 5 % glutaraldehyde, dehydrated in 70 % ethanol and 70 % acetone and stored in pure acetone for at least two days before being dried by the critical point technique (Andersson 1951) After being coated with gold in a "sputter", the specimens were examined in a JSM U 3 microscope

The vascular anatomy was studied after injection of silicone rubber (Canton Bio Medical Products Inc, Box 2017, Boulder Colorado 80302, USA) into the blood vessels (Nordin & Lindholm, 1977 a) After micro dissection and dehydration the specimens were immersed in glycerol or methyl salicylate which rendered them transparent and permitted excellent visualization of the blood vessels of the tracheal wall

For light microscopy and TEM two methods were used With the first technique the specimens were fixed in buffered 2 5 % glutaraldehyde post fixed in 1 5 % osmium tetroxide for 1 5 h and embedded in Epon 812 (Luft, 1961) With the second technique the specimens used for SEM were immersed for 24 h in propylene oxide and embedded in Epon 812 The specimens for light microscopy and TEM were sectioned in an LKB Ultrotome The TEM specimens were studied in a Siemens Elmiskop IA

Intubation tube and cuff The rabbits were anesthetized by an intravenous injection of 2 % sodium pentobarbital (Nembutal®) in a dose of 50–60 mg/kg body weight They breathed spontaneously throughout the experiment A specially made PVC tube (Portex) with an inner diameter (ID) of 2 5 mm and an outer diameter (OD) of 3 5 mm, equipped with a large cuff was placed in the trachea by translaryngeal intubation The thickness of the cuff wall varied slightly around 0 10 mm depending upon the degree of prestreching (Geffin & Pontoppidan 1969) When the cuff was deflated the diameter of the tube and cuff varied between 4 2 and 3 9 mm depending upon the folds in the cuff The length of the cuff was approximately 15 mm The diameter of the trachea was about 5 mm

The tube, with the cuff deflated, was placed in the cervical portion of the trachea, well below the larynx, and left in place for 15 min either uninflated or inflated to different cuff pressures as measured according to Cox & Schatz (1974) By measuring the pressure in a cuff with large diameter, high volume characteristics the C T pressure is also known, as they are identical so long as there is no circumferential tension in the cuff wall (Carroll et al, 1974) The animals were killed directly after this procedure by injection of Nembutal® and air

Results

The normal tracheal wall

The normal tracheal wall was found to consist of a mucus membrane and a series of horse shoe shaped cartilages of the same appearance as in the human trachea The surface layer of the mucosa is built up of a *pseudostratified columnar ciliated epithelium* containing many goblet cells This rests on a rather thick (1000 Å) basement membrane, beneath which is a loose network of connective tissue cells and fibers forming the lamina propria In this layer there is a rich capillary network

The submucosal layer also contains loose con nective tissue and rather large groups of tubulo alveolar sero-mucus glands These glands and the goblet cells together produce the mucus coating the tracheal wall The ciliated cells are very numerous and cover a large part of the tracheal surface In scanning electron micrographs large areas of tracheal mucosa may be surveyed (Fig 1) and it is also fairly easy to differentiate between ciliated and goblet cells The combined use of SEM and TEM (Fig 2) gives excellent informa tion on the structure of the tracheal mucosa Each ciliated cell extends from the basement membrane to the epithelial surface These cells have an ovoid nucleus located in their lower half, and contain an endoplasmic reticulum, a large number of mitochondria and other cytoplasmic organelles The free upper surface of the cells is provided with large numbers of cilia the number varying with the area of the free surface Between the cilia there are numerous microvili The cilia are 6–7 μm long and approximately 0 2 μm in diameter They taper slightly at the distal end and have an axial [illegible] The outer membrane of the cilium is continuous w[illegible] the plasma membrane of the cell The axial fi[illegible] ment complex generally consists of 11 inter[illegible] fibrils arranged with two in the center and n[illegible] surrounding evenly spaced double fibrils (Fig[illegible] As will be shown in this paper cilia with[illegible] deviating arrangement in the axial filament co[illegible] plex are often found, especially in tracheal muc[illegible] affected by different forms of lesions. Also "normal" tracheae cilia with quite differing int[illegible] nal structures may be seen, as well as some gi[illegible] cilia Giant cilia seem to be more numerous damaged cells (Fig 7 b)

Towards the tip of the cilium the nine periphe[illegible] double fibrils become single and a reduction number is often evident Each cilium has a ba[illegible] body and a tapering, cross-striated rootlet at [illegible] cell surface The rootlet can be followed far do[illegible] into the cell

The goblet cells or mucus producing cells [illegible] quite different in appearance from the cilia[illegible] cells, being goblet shaped with an upper wide[illegible] portion and a slender foot The nucleus is loca[illegible] in the lower part of the cell There is a [illegible] developed Golgi complex close to the nucleus [illegible] assumed that secretory granules are formed in [illegible] region and that these fill the upper goblet sha[illegible] or thecal portion of the cells At TEM and li[illegible] microscopy these granules are round rather la[illegible] and densely packed The cytoplasm conta[illegible] many ribosomes, and also often numer[illegible] lysosomes The free surface of the cells is of[illegible] richly provided with microvili The cytopla[illegible] contains numerous mitochondria and at the ba[illegible] end a rough endoplasmic reticulum



Fig 1 Scanning electron micrograph of the normal tracheal epithelium of the rabbit with many ciliated and a few non ciliated cells with microvilli ×5 100

The tracheal wall after intubation

Generally the major damage to the tracheal wall after intubation is caused by the cuff though minor injury may be caused by the endotracheal tube In the following we shall only deal with damage caused by the cuff The intubation time was kept constant at 15 min

When the tube was placed in the trachea without inflation of the cuff it was quite clear that this was sufficient to cause minor superficial damage to the epithelial lamina At SEM it was evident that this damage only occurred over regions where a car tilage was situated In the intercartilaginous regions no damage was seen (Fig 4) In the in jured regions TEM revealed that the ciliated cells had largely lost their cilia the basal bodies were sometimes present however, and close to the sur face the cytoplasm was vacuolated

In this series the damage was found not to have penetrated the basement membrane The normal tracheal epithelium has been described above as rather high and pseudostratified Close to areas of *damage we repeatedly found regions still retaining* cilia with a fairly normal appearance but the thickness of the epithelium had become reduced In these regions two layers or a single layer of ciliated cells were seen How this modification develops is not clear but it may be assumed that the pressure in some way promotes a lateral move ment of the cells away from the center of maximal damage (Fig 5) Another feature was that in regions of incipient damage microvilli between the *kinocilia had started to disappear*



Fig. 2a. Scanning electron micrograph of normal tracheal mucosa from the rabbit. The tracheal cilia and the interciliary microvilli are clearly seen. × 7 300
Fig. 2b. Transmission electron micrograph from the same part of the mucosa as in *a*. × 34 500

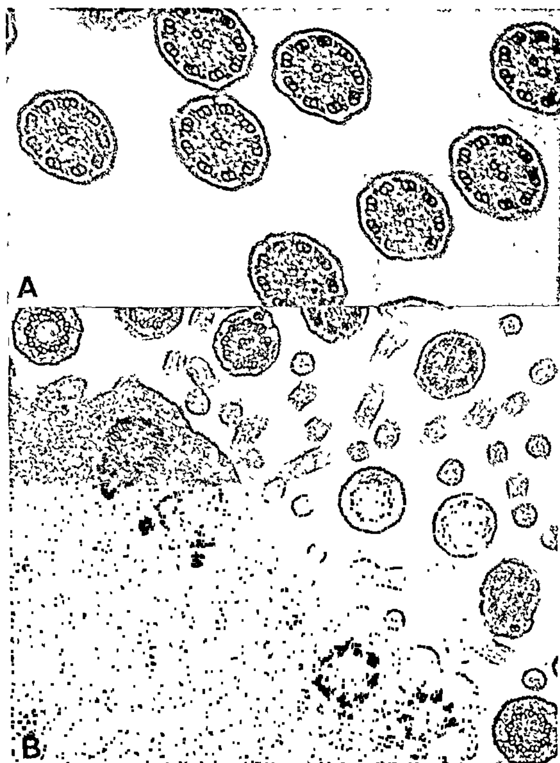


Fig 3a Cross sectioned tracheal cilia In each cilium nine double fibrils are surrounding two central single fibrils × 126 000

Fig 3b Cilia close to and below the cell surface A comparison between *a* and *b* shows that the cilia change their structure when entering the cell surface Both from normal rabbit mucosa × 92 000



Fig. 4. Minor mucosal damage to a rabbit trachea after 15 min of intubation but without inflation of the cuff. Superficial damage *(arrow)* can be seen over the tracheal cartilage (C). ×70

Inflation of the cuff resulted in an increase of the mucosal damage, the extent of the injury being directly related to the pressure in the cuff. A gradual increase in C.T. pressure thus led to progressive extension of the mucosal damage. This took the form of both widening of the injured areas and penetration of the damage to deeper regions. Often complete denudation but no penetration of the basement membrane was seen with only some scattered cells remaining. Regions with almost no epithelial cells lying close to regions with almost normal epithelium were commonly found. Fig. 6 shows how *C.T. pressures of 20 or 30 mmHg* caused fairly deep injuries but no penetration of the basement membrane. In the center of the damaged area in Fig. 6 b the ulceration reached the basement membrane and almost all cells were lost. Close to this more severely damaged area partly disintegrated cells and cells with cytoplasmic vacuolization and partly destroyed cilia were seen (Fig. 7 b).

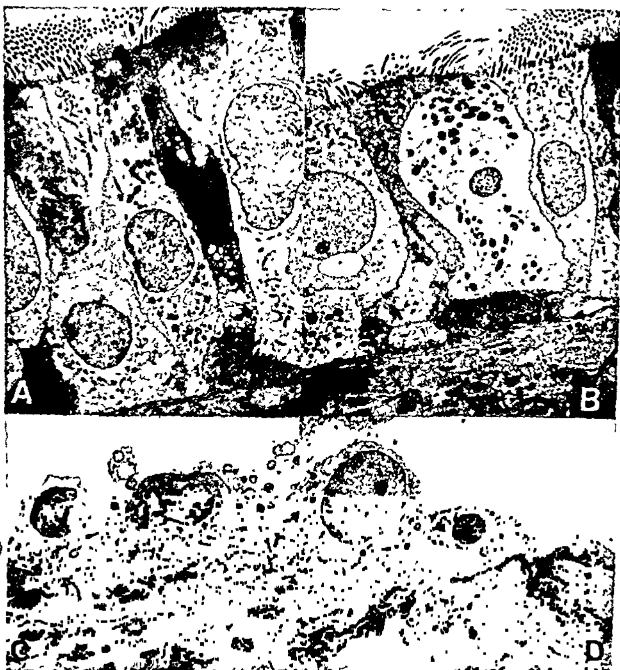


Fig 5 In these four transmission electron micrographs from rabbit tracheae exposed to a C-T pressure of 100 mmHg for 15 min, it is seen how fairly adjacent regions contain cells differing greatly in appearance Some cells *(a)* are only slightly modified, others are forming a low epithelium *(b)* and a third group *(c, d)* are forming only scattered, degenerating cells at the basement membrane *(a)* ×3 100 *(b)* ×3 100 *(c)* ×3 200 *(d)* ×3 300

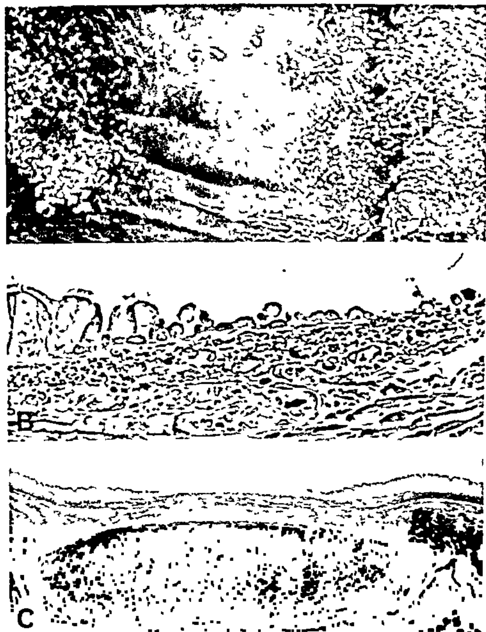


Fig 6 Rabbit tracheal mucosa exposed to a C T pressure of 20 mmHg for 15 min showing incipient degeneration over the cartilages In *a* a scanning electron micrograph shows the dark areas of degeneration *(arrow)* In *b*, phase contrast microscopy reveals the destruction of the epithelium in a damaged area In *c* a corresponding area at low magnification is seen The damage over the tracheal cartilage is very clear *(a)* × 180 *(b)* × 600 *(c)* × 50



Fig 7. Transmission electron micrographs from two adjacent regions of a rabbit trachea exposed to a C-T pressure of 30 mmHg for 15 min In *a* the cells and the cilia are well preserved ×8 900 In *b* the cells seem to be degenerating One triple cilium can be seen *(arrow)*, possibly a result of fusion ×9 100



Fig. 8. Scanning electron micrographs showing rather deep damage *(arrow)* of the tracheal epithelium from a rabbit exposed to a CT pressure of 50 mmHg for 15 min. In *b* it can be seen how the basement membrane is almost devoid of epithelial cells. *(a)* ×70. *(b)* ×450.



Fig 9 Further extension of the mucosal damage *(arrow)* in a rabbit trachea exposed to a C-T pressure of 80 mmHg for 15 min The damage is most pronounced over the cartilages ×80

Fig 7 a shows that only a few cells away from where Fig 7 b was taken, well ciliated cells were present It seemed as if the cells were able to withstand a certain pressure but when the surface structures became damaged the cells rapidly disintegrated The impact of infection could not be evaluated, but as the intubation time was only 15 min infection could not have progressed very deeply Some lympholeucocytic reaction was observed, however, even at this stage (Fig 7 b)

With an increase of the *C T pressure to 50–60 mmHg* the damage was slightly greater but the injured areas were found mainly over the cartilages, while the epithelium in the regions between them seemed to be hardly affected (Fig 8 a) In the scanning electron micrographs in Fig 8 b it can be seen how the injured regions were almost devoid of cells and the basement membrane was practically denuded The intercartilaginous regions, however, and the membranous portion of the tracheal wall retained a fairly normal appearance When the *C T pressure exceeded 80 mmHg* the major damage was still found over the cartilaginous portions but now the injuries were more extensive, widening irregularly and invading intercartilaginous regions (Fig. 9) At a *C T pressure of 100 mmHg* the damage afflicted almost the entire mucosa and only small undamaged mucosal regions remained At this stage it appeared as if the basement membrane had also begun to disintegrate (Fig 10).

The vascular bed of the tracheal wall

The vascular bed of the tracheal wall has been described by Nordin & Lindholm (1977 a) The main principles of the vascular organization are illustrated in Fig 11 The effect of an inflated large cuff on the vascular bed is shown in Fig 12.



Fig 10 In *a* a scanning electron micrograph shows extensive damage *(arrows)* to a rabbit trachea exposed to a C T pressure of 100 mmHg for 15 min ×60 In *b* and *c* more or less complete denuda t on of the basement membrane is seen There also seems to be some disintegration of the basement membrane *(b)* *(b)* ×900 *(c)* ×900

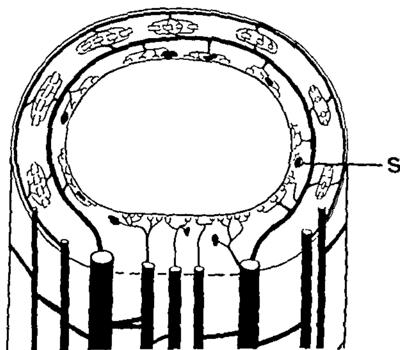


Fig 11 Schematic drawing showing the vascular anatomy of the rabbit tracheal wall S = sinusoids or cavernous vascular spaces in the sub mucosa

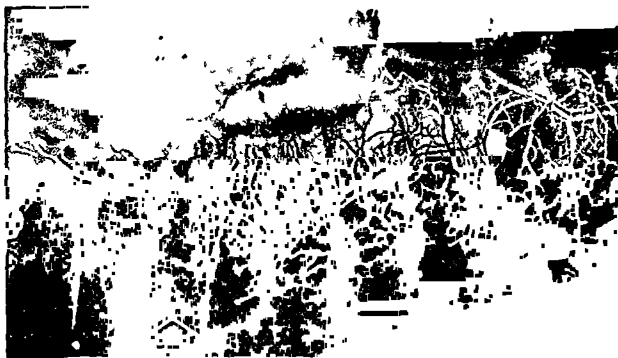


Fig 12 Cat tracheal mucosa with silicone rubber infused into the vessels The cuff was inflated during the infusion The arrow shows the borderline between the cuffed part of the mucosa with no silicone rubber in the vessels (to the left) and the uncuffed part with vessels filled with silicone rubber (to the right) At the cuffed area the mucosal vessels are obviously being occluded by pressure from the inflated cuff

Discussion

The fine structure of the tracheal epithelium has been extensively studied by several authors, both by TEM (Rhodin, 1966, Konradova, 1966, Mecklenburg et al, 1974) and by SEM (Barber & Boyde, 1968, Holma, 1969, Dalgren et al, 1972, Mecklenburg et al, 1974)

Rhodin (1966) distinguished at least three different cell types in the tracheal epithelium At the basement membrane basal cells with a rather large nucleus form an irregular layer and ciliated cells and goblet cells form the upper portion of the epithelial lamina The ciliated cells predominate Many authors have distinguished a further group of intermediate cells, which reach from the base ment membrane to the upper half of the epithelial layer For the present study the ciliated cells were of special interest as they are the structures in im mediate contact with the endotracheal tube Rhodin (1966) estimated the number of cilia per cell at about 200 but we have seen cells with more than 1000 cilia and also cells with fewer than 200 Even in normal' tracheal epithelium cilia with quite differing internal structures may be seen as well as some giant cilia Giant cilia (Fig 7 b) are more numerous in damaged areas

The reaction of the subepithelial tissues to in tubation is also of great importance This is e specially true for the vascular bed and the hyaline tracheal cartilages These cartilages with their well developed perichondrium form elastic horse shoe shaped reinforcements in the tracheal wall and their function is of mandatory importance, to pre vent the trachea from collapsing

Numerous studies have been reported con cerning the damage caused by intubation but in many of them the small high pressure cuff has been employed. In such an experimental set up the C T pressure is extremely difficult to ascertain Clear evidence has now been produced that large cuffs are less injurious

In our studies the cuffs were prestretched (Gef fin & Pontoppidan, 1969) to obtain large resting diameter, large-residual volume cuff properties (Lomholt, 1967, Carroll & Grenvik, 1973, Carroll et al, 1974) in relation to the rabbit trachea The cuff wall, however, proved to be not sufficiently thin and pliable to possess the proper ties of an *ideal* large cuff The ideal large cuff should drape freely over the mucosa, allowing the C T pressure to be distributed evenly over this sur face, including the mucosa covering the intercar tilaginous regions

Our results have been influenced by the fact that the wall of the cuff was rather thick in relation to the thickness of the rabbit tracheal mucosa This fact should be borne in mind when inter preting the results Although the resting diameter and residual volume were large enough for it to be classified as a large cuff, the pressure dynamics against the rabbit tracheal wall resembled at least partly those of a small cuff, due to the rigidity and relative thickness of the cuff wall

It is quite clear from our experiments that even an uninflated cuff of an endotracheal tube damages the surface of the tracheal mucosa This could be expected from the early experiments by Hilding (1952), and this question was further dis cussed in his more recent studies (1971) Our observation is also in accordance with the findings of Klainer et al (1975) The shearing forces of the cuff at intubation and extubation probably con tribute to the lesions even if the cuff is completely

deflated during these procedures and great care is taken to avoid surface damage to the airway It is also quite clear that if the period of intubation had been longer than 15 min, or if there had been significant movement of the rabbit's head, then the shearing forces may have become a more significant factor

In the present experiments some areas, especially on the mucosa covering the tracheal cartilages, lost their cilia and in small areas ciliated cells also disappeared These lesions were rather scattered, however, and did not cover large confluent areas From a functional point of view this might mean that the capacity for mucus transport along the trachea is reduced but not completely eliminated

From analyses of tube deformation forces (Lindholm, 1969, Lindholm & Carroll, 1975) exerted on the airway by a tube of conventional shape, it is evident that on intubation and extubation the tip of the tube is likely to scratch the anterior tracheal mucosa and the force applied posteriorly may also play a certain role This was also emphasized by Hilding (1971) Such lesions may occasionally interfere with the interpretation of cuff damage but in this analysis we have little reason to believe that this is the case

We are convinced that when it is reported that no or only minor damage to the tracheal mucosa has been found at investigations similar to the present one, the explanation is that SEM has not been used This method permits detailed observation of large areas of the mucosa and allows selection of the most severely damaged areas for light microscopy and TEM, which will give further information on the ultrastructure of the specific lesions Such lesions may otherwise be overlooked

It is also evident from the present study that the C T pressure is of major significance for the extent and depth of the damage caused by the cuff As stated above, however, even an uninflated cuff causes a certain amount of damage, but our experiments also clearly demonstrate that C T pressure and the magnitude of damage are directly related At ordinary C T pressures of 15–20 mmHg for 15 min the surface lesions may not differ greatly from those caused by shearing forces of a deflated cuff at intubation At higher C T pressures the epithelial lesions become deeper and more confluent The majority of the epithelial cells at the cuff site are thus destroyed at C T pressures of 80–100 mmHg or more At this stage it appeared as if the basement membrane had also begun to disintegrate In this connection it is to be noted that all small slip on tracheostomy cuffs tested by Carroll et al (1969) exerted a C T pressure of 130–147 mmHg for minimum no leak ventilation

In addition to C T pressure the time factor is of importance in the development of tracheal wall damage This has been further elucidated in another study (Nordin et al , 1977 b), which is also reported in this supplement

We have just described how in our experiments the surface epithelium disappeared in some areas, but in others was only compressed After a short intubation period the basement membrane was, however, usually retained This membrane constitutes an important protective layer against severe damage In some of our animals it was slightly disintegrated In such cases an invasion of lympholeucocytic cells close to the membrane and even into the remaining epithelial border regions was sometimes seen

It was also quite clear that the damage began and was most pronounced at the mucosa covering the tracheal cartilages This is explained by the fact that the wall of the cuff used was not sufficiently thin and pliable to have the properties of an ideal large cuff, in relation to the smallness of the rabbit trachea The ideal large cuff should drape quite freely over the mucosa, allowing the C T pressure to be distributed evenly, i e also to a certain degree, to the intercartilaginous areas Our cuffs did not quite possess this property, as they often left this part of the mucosa untouched, even when inflated to fairly high C T pressures This emphasizes the importance of the quality of the cuff wall The thicker and the more rigid it is, the more will the cuff act as a small volume type i e it will ride on the edges of the cartilages This will increase the risk of local ulceration, which may penetrate the basement membrane at the mucosa covering the cartilages and eventually threaten the cartilages themselves In contrast commercially

available, extremely thin walled large cuffs, used in 72 h intubation tests on dogs, have seemed to be in contact with the entire mucosa at the cuff site, even when inflated to only 15 mm Hg (Lindholm et al, 1977) This means that there are some thin walled large cuffs available today that more closely approach the ideal cuff than the cuff used in our experiments

The results of our studies of the effect of C T pressure on the blood perfusion of the mucosa in contact with the cuff used (Nordin et al 1977 d) revealed that some blood perfusion was still taking place even when the calculated perfusion pressure was zero or even negative, suggesting that the cuff had not transmitted the entire C T pressure to all parts of the mucosa at "cuff level" and thus allowed the blood perfusion to persist in some intercartilaginous spaces It is concluded that if the wall of a large cuff is very thin and pliable the blood circulation in the mucosa may continue at least for some time, even at C T pressures exceeding 30 mmHg (approximate maximum capillary pressure in the tracheal mucosa) The same study also indicated that a non ideal cuff probably stops the circulation in the mucosa covering the cartilages at C-T pressures exceeding 30 mmHg

A large cuff with a thin and pliable wall will distribute the cuff pressure over a large area of tracheal mucosa and will seal at a low C T pressure It must be emphasized, however, that the use of large cuffs at high pressures allows the high intracuff pressure to be transmitted undiminished until such time as the diameter of the trachea exceeds the diameter of the cuff, and thus circumferential tension within the cuff starts to limit the effective C T pressure At such a time, the trachea is already dilated, and the physics of seal become the physics of small cuff (high pressure) sealing, which were elaborated by Carroll et al (1974)

In order to avoid overinflation of the large cuff, the intracuff pressure (= C T pressure) should always be measured by means of a four way stopcock and an aneroid manometer In the case of extended periods of mechanical ventilation with high airway pressure, the resulting tracheal diameter at the cuff site should be checked radiographically

Conclusions

1 Any tracheal intubation will produce surface lesions of the mucosa

2 The degree of damage is directly related to the C T pressure

3 In this study C T pressures of 20–30 mmHg sometimes produced small areas of lesions penetrating the epithelial layer after 15 min of in tubation

4 C T pressures above 50 mmHg often produced widespread areas of destroyed epithelium, leaving the basement membrane visible

5 The basement membrane, on the other hand, seemed to constitute a good protection against deeper penetration of the lesion, not being dis integrated until C T pressures of about 100 mmHg for 15 min

6 A thick walled large cuff tends to give lesions similar to those seen after the use of a small cuff

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Zusammenfassung

e Wirkung einer grossen Schlauchmanschette f die Schleimhaut der Kaninchenluftröhre wur mit sowohl Fasenkontrastmikroskopie als aus anning (SEM) und Transmissionselektronen kroskopie (TEM) untersucht Der Schlauch lag Minuten in der Trachea Die Manschette war tweder unaufgeblasen oder wurde auf "cuff to icheal wall" Drucke bis zu 100 mm Hg aufgeb sen

Die unaufgeblasene Manschette verursachte berflächenschäden der Epithelschicht in Regio n mit Knorpelunterlage

War die Manschette aufgeblasen, kam es zu ei r vermehrten Schleimhautschädigung, deren usmass direkt vom Manschettendruck abhängig ar Sie äusserte sich sowohl in einer Erweiterung :s betroffenen Abschnittes als auch in einer :hadigung tieferer Schichten Bei einem C T ruck von 100 mm Hg war fast die gesamte :hleimhaut geschadigt Nur kleine Schleimhaut)snitte blieben unversehrt In diesem Stadium :igte auch die Basalmembran Anzeichen begin :nden Zerfalles

Es ist bekannt dass eine *kleine* Schlauchman :hette leicht zu tiefgreifenden Ulzerationen er Schleimhaut uber dem Knorpel führt

Aus dieser Untersuchung geht hervor, dass eine *grosse* Schlauchmanschette zu einer ebenartigen Form von Ulzerationen führt, wenn einerseits die Manschettenwandung nicht genügend dünn und geschmeidig ist und wenn andereseits die Manschette derart aufgeblasen wird, dass die Trachea zu einer Weite ausgedehnt wird, die den Durchmesser der Manschette übertrifft Es kommt gleichzeitig zu einer Peripheriespannung in der Manschette und damit zum gleichen physicalischen Abdichtungseffekt wie bei der kleinen Manschette mit hohem Druck

Eine grosse, mit entsprechender Sorgfalt eingeführte Schlauchmanschette ist für die Luftröhre schonender als eine kleine Manschette Um ein übermässiges Aufblasen der Manschette zu vermeiden, sollte der Innendruck der Manschette (= C T pressure) mit Hilfe eines Anaeroidmanometers über einen Vierweghahn gemessen werden Wenn uber längere Zeit mechanische Ventilation mit hohem Luftwegedruck betrieben wird, sollte der dabei verursachte Durchmesser der Luftröhre mit dem Situs der Schlauchmanschette röntgenologisch kontrolliert werden

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Surface structure of the tracheal wall after different durations of intubation

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Abstract The effect of a large tracheal tube cuff on the rabbit tracheal mucosa was investigated by phase contrast microscopy and scanning (SEM) and transmission (TEM) electron microscopy

The effect of different durations of intubation on the extent of the damage was studied the intubation time being varied between 1 and 4 hours

The results were compared with those of a previous study in which the effect of different cuff to tracheal wall pressures (C T pressures) on the extent of mucosal damage was investigated

It was found that of these two factors the C T pressure has the greatest *relative* importance

It is concluded that if the C T pressure is kept low (below 20 mmHg), then the trachea is able to resist even a long duration of cuffed intubation with use of a large thin walled cuff

Introduction

Tracheal intubation over prolonged periods sometimes causes sufficient damage to the tracheal wall to result in a clinically significant tracheal stenosis Incidences varying from 1 5 to 20% have been reported (Pearson et al, 1968, Lindholm, 1969, Grillo, 1970) Geffin & Pontoppidan (1969) concluded that the stenosis most often was found in the region where the cuff had exerted its main pressure against the tracheal mucosa The most widely used cuffs have been of the small resting diameter, small resting volume type, subsequently called *small* cuffs Many publications have described the damage caused by this type of cuff and most authors have come to the conclusion that the main etiological factor in this damage is the high pressure exerted on the tracheal wall (Pearson et al, 1968, Cooper & Grillo, 1969, Shelly et al, 1969, Donnelly, 1969, Hedden et al, 1969, Grillo et al, 1971, Andrews & Pearson, 1971, Hilding, 1971, Magovern et al, 1972, Goldenberg & Pearson, 1972)

Further analyses of the cuff problem were made simultaneously by many research groups (Lomholt, 1967, Bryce et al, 1968, Carroll et al, 1969, Hedden et al, 1969, Cooper & Grillo 1969, Geffin & Pontoppidan, 1969) The conclusion from these investigations was that the small cuff causes uncontrollable pressure against the tracheal wall and that it is impossible to measure the C T pressure by recording the intracuff pressure

The large resting diameter, large residual volume type of cuff (subsequently called the *large* cuff) was proposed by Lomholt (1967) Carroll et al. (1969), Cooper & Grillo (1969)

Geffin & Pontoppidan (1969) "prestretched small plastic cuffs and thus converted them to large cuffs All these groups found that the large cuff was able to "seal" at a much lower C T pressure than the small cuff

Several convincing reports have since demonstrated that cuff site tracheal injury is lower with large than with small cuffs (Cooper & Grillo, 1969, Grillo et al, 1971, Ching et al, 1971, Dunn et al, 1974, Mathias & Wedley, 1974, Paegle & Bernhard, 1975) This made us focus our interest on the *large* cuff in the present investigation and no comparative studies with small cuffs were included

The aim of this investigation was to study tracheal damage caused by different durations of intubation, keeping the C T pressure constant

In another investigation (Nordin et al, 1977a) the tracheal damage caused by different C T pressures was studied, keeping the duration of intubation constant

Through these two investigations an attempt was made to answer the question of which is the most important factor in causing mucosal damage, the C T pressure or the duration of intubation Another aim was to create a model by which "standardized" tracheal damage of different degrees could be produced to allow a study of the regeneration of mucosa injured to a fairly well controlled depth This study is in progress at present

In parallel, we have studied

1) the vascular anatomy of the tracheal mucosa by arterial infusion of silicone rubber, and the effect of an inflated large cuff on the vascular bed (Nordin & Lindholm 1977 b)

2) the capillary blood perfusion pressure and fluid balance of the tracheal mucosa by the micropuncture technique (Nordin et al, 1977 c),

3) the effect of different C T pressures on the capillary blood flow of the tracheal mucosa (Nordin et al, 1977 d)

Material and methods

Fifteen white rabbits, weighing between 2 0 and 3 4 kg, were used

The structure of the tracheal wall was examined by direct observation—using a binocular preparation microscope (Wild M 5), and by phase contrast microscopy and scanning (SEM) and transmission (TEM) electron microscopy The techniques for SEM and TEM were those currently used in our clinic (Ades & Engstrom, 1972) For SEM the specimens were fixed in buffered 2 5 % glutaraldehyde, dehydrated in 70 % ethanol and 70 % acetone and stored in pure acetone for at least two days before being dried by the critical point technique (Andersson 1951) After being coated with gold in a sputtering system, the specimens were examined in a JSM U 3 microscope

For light microscopy and TEM two methods were used With the first technique the specimens were fixed in buffered 2 5 % glutaraldehyde, post fixed in 1 5 % osmium tetroxide for 1 5 h and embedded in Epon 812 (Luft, 1961) With the second technique the specimens used for SEM were immersed for 24 h in propylene oxide and embedded in Epon 812 The specimens for light microscopy and TEM were sectioned in an LKB Ultrotome The TEM specimens were studied in a Siemens Elmiskop IA

Intubation tube and cuff The rabbits were anesthetized by an intravenous injection of 2 % sodium pentobarbital (Nembutal®) in a dose of 50—60 mg/kg body weight They breathed spontaneously throughout the experiment A specially made PVC tube (Portex) with an inner diameter (ID) of 2 5 mm and an outer diameter (OD) of 3 5 mm was placed in the trachea by translaryngeal intubation The cuff had been "prestretched" (Geffin & Pontoppidan, 1969) to obtain large-cuff properties (Lomholt, 1967, Carroll & Grenvik, 1973), in relation to the rabbit trachea The thickness of the cuff wall varied slightly around 0 10 mm depending upon the degree of prestretching When the cuff was deflated the diameter of the tube and cuff varied between 4 2 and 3 9 mm depending upon folds in the cuff The length of the cuff was approximately 15 mm The diameter of the trachea was about 5 mm

The tube, with the cuff deflated, was placed in the cervical portion of the trachea, well below the larynx The time of intubation was varied between 1 and 4 h The results are presented in three different groups of C T pressures, with variations of the intubation time within each group The C T pressure in the first group was fairly "normal", 20 mmHg in the second group "medium", 50 mmHg, and in the third group "high", 100 mmHg The pressures were measured according to Cox & Schatz (1974) By measuring the pressure in a cuff with large cuff properties the C T pressure is also known, being identical (Carroll et al , 1974). The animals were killed directly after the decided time of intubation by injection of Nembutal® and air

Results

At a C T pressure of 20 mmHg for *1 to 2 h* of intubation the mucosa on top of the cartilages was damaged to such an extent that it was partly denuded almost down to the basement membrane (Fig 1 a) In this region about 50 % of the epithelial cells still remained, though they were damaged and somewhat compressed (Fig 1 b, left)

In intercartilaginous regions most of the epithelium seemed well preserved, as seen at a low magnification (Fig 1 a) At higher magnification it was obvious that some of these cells had lost their cilia, partly or completely (Fig 1 c) TEM revealed that in some areas there was also incipient disintegration of the interior of the epithelial cells, with migration of leucocytes up to the basement membrane (Fig 2 b)

At *3 h* of intubation the damage was more widespread (Fig 3 a) and the epithelium was now partly compressed in the intercartilaginous areas also (Fig 3 b) Areas approaching the top of the cartilages showed an increasing degree of damage, varying from partly denuded epithelium to a complete loss of epithelial cells and a denuded basement membrane (Fig 3 c) In the figure some erythrocytes are visible

At a C T pressure of 50 mmHg for *2 h* of intubation most of the epithelial cells in the mucosa on top of the cartilages were destroyed or compressed The basement membrane seemed intact but partly denuded (Fig 4 a) The severely damaged areas on top of the cartilages were now wider compared with those at a C T pressure of 20 mmHg At *4 h* of intubation some parts of the mucosa in the intercartilaginous regions consisted of low epithelial cells covered by microvilli These cells were intermingled with normally ciliated cells (Fig 4 b)

At a C T pressure of 100 mmHg for *1 h* of intubation the entire mucosa at cuff level was damaged to some extent Small intercartilaginous areas of the mucosa were fairly well preserved, however, the cilia being only slightly compressed On top of the cartilages, on the other hand wide parts of the basement membrane were denuded and in some areas this was completely absent, leaving the mucosal stroma visible At *2 h* of intubation intercartilaginous areas of the mucosa had lost their cilia and the epithelial cells were separated from each other At *4 h* of intubation the mucosa covering the cartilages was sometimes destroyed down to the cartilage itself, and bacteria were found for the first time to be invading the damaged mucosa (Fig 5)



Fig 1 Scanning electron micrographs of rabbit tracheal mucosa exposed to a C T pressure of 20 mmHg for 1—2 hours *a* At a low magnification (×80) it is seen that the mucosa on top of the cartilage is most severely damaged *(arrows)* *b* The most severely damaged area overlying the cartilage (to the left) seen at a higher magnification (×800) In this region about 50% of the epithelial cells *still remain The intercartilaginous region (to the right) seems fairly well preserved*, but a higher magnification (×1 700) in this area *(c)* shows that some of the cells have lost their cilia, partly or completely



Fig 2 Transmission electron micrographs from rabbit tracheal mucosa *a* Normal mucosa with ciliated cells and goblet cells (×4 200) *b* Mucosa exposed to a C-T pressure of 20 mmHg for 2 hours In the most severely damaged area (to the right) most of the epithelial cells have sloughed off At the borderline against the better preserved part of the mucosa (to the left in the photograph) there is incipient disintegration of the interior of the epithelial cells with migration o leucocytes (*) up to the basement membrane (×3 000)



Fig. 3. Scanning electron micrographs of rabbit tracheal mucosa exposed to a C.T. pressure of 20 mmHg for 3 hours. *(a)* The damage here is more widespread than after 2 hours of intubation. The epithelium is now partly compressed in the intercartilaginous areas also. *(b)* In more severely damaged areas only scattered epithelial cells remain and the basement membrane is partly denuded. *(c)* *(a)* ×60, *(b)* ×900, *(c)* ×700.



Fig 4 Scanning electron micrographs of rabbit tracheal mucosa *a* Mucosa exposed to a C-T pressure of 50 mmHg for 2 hours Most of the epithelial cells in the mucosa on top of the cartilages have been destroyed or compressed The basement membrane seems to be intact (× 1 700) *b* Intercartilaginous region of a mucosa exposed to 50 mmHg for 4 hours Among normally ciliated epithelial cells, low epithelial cells covered by microvilli are seen Whether this is a sign of incipient disintegration of the cilia or a sign of incipient cilial regeneration cannot be decided (× 3 000)

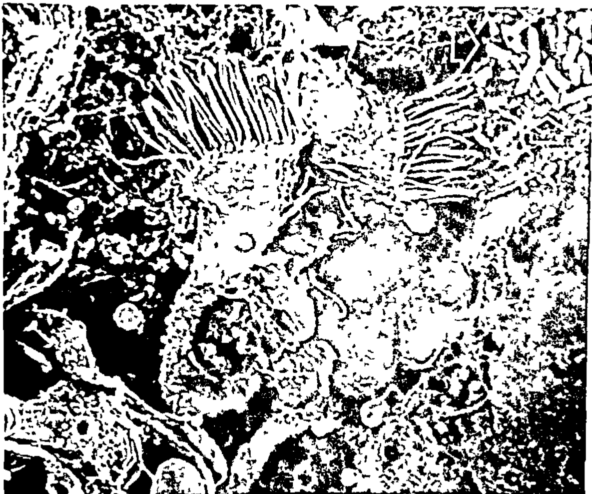


Fig 5 Scanning electron micrograph of rabbit tracheal mucosa exposed to a C T pressure of 100 mmHg for 4 hours The exposed mucosal stroma is—the only time in all our experiments—invaded with bacteria *(arrows)* (× 4 000)

Discussion

A C T pressure of 20 mmHg for *up to 2 h* of intubation did not cause significantly more damage than was found after 15 min of intubation at the same pressure, in another investigation (Nordin et al 1977 a) In intercartilaginous regions in the present study some epithelial cells were still preserved but the mucosa seemed more swollen (Fig 1 a) than after 15 min of intubation

A C T pressure of 50 mmHg for *up to 2 h* of intubation did not seem to cause significantly greater damage than when this pressure was applied during 15 min of intubation The basement membrane seemed intact but partly denuded (Fig 4 a) At *4 h* of intubation some parts of the mucosa in the intercartilaginous regions consisted of low epithelial cells covered by microvilli These cells were intermingled with normally ciliated cells Whether this was an expression of incipient disintegration of the cilia or a sign of incipient cilial regeneration only 4 h after the damage was produced cannot be answered at present This question is being examined further in an investigation now in progress (Nordin) where the regeneration of cuff induced mucosal damage is being studied

C T pressure of 100 mmHg At this pressure it seemed in the previous investigation (Nordin et al 1977 a) as if the basement membrane had begun to disintegrate after only 15 min of intubation In the present study it was seen that after *1 h* of intubation the damage was more serious with areas in which the basement membrane was completely absent leaving the mucosal stroma exposed At *4 h* of intubation the damage sometimes penetrated almost down to the cartilage and considerable bacterial invasion of damaged mucosa was found This was the only time in all our experiments at which bacteria were observed It thus seems that even with fairly penetrating damage local infection does not begin to play a serious role until after about four hours of intubation There is little reason to believe that the bacteria arrived there by contamination as this *never* happened with less severely damaged animals

In our opinion the C T pressure is more important than the duration of intubation for the occurrence of tracheal damage This is certainly true for ordinary periods of intubation i e up to three hours This finding is in accordance with the observations reported by Mathias & Wedley (1974)

Conclusions

1. Tracheal cuff damage is highly influenced by the product of C T pressure and duration of intubation

2 Of these factors, C T pressure has the greatest importance

3 This means that if the C T pressure is kept low, then the trachea is able to resist even a long duration of cuffed intubation

4 In addition to the above mentioned traumatizing factors, other factors are also of importance These include the shape of the tube and cuff physical and chemical properties of the tube and cuff material, local infection and the general condition of the patient

5 To diminish tracheal lesions the product of all traumatizing factors should be kept as low as possible The anesthetist should pay special attention to the following

I A high quality tube which will easily adapt to the anatomy of the airway should be chosen, and should be equipped with a thin walled large cuff

II Recordings should be made to ensure a C T pressure below 20 mmHg

III Movements of the cuff relative to the tracheal wall should be minimized

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SUPPLEMENT 346

A Mathematical Model of the Vestibulo-Oculomotor Reflex in Physiological and Pathological Conditions

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SUPPLEMENT 346

A Mathematical Model of the Vestibulo-Oculomotor Reflex in Physiological and Pathological Conditions

BY

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Introduction

Several authors (Steer et al., 1968, Malcom, 1968, Young, 1969, Young & Oman, 1969, Outerbridge, 1969, Sugie & Jones, 1971, Barnes & Benson, 1973) have tried mathematically to implement the vestibulo-oculomotor reflex by proposing models capable of reproducing the main experimental data. However, important types of phenomena such as vestibular adaptation and secondary nystagmus cannot be justified by some of these models. Other authors (Young & Oman 1968, Malcom & Jones, 1970) have tried to reproduce the effects of short-term vestibular adaptation by improving the simple second-order torsion pendulum model of Steinhausen (1931) accounting for the mechanical behaviour of the cupula-endolymph system. Only Fernandez & Goldberg (Fernandez & Goldberg, 1971, Goldberg & Fernandez, 1971*a b*) on the basis of their experimental findings were able to describe mathematically the cupular receptors' mechano-neural transduction. However these models do not suggest any neural mechanism apt to reproduce nystagmic beats. More recently a model overcoming these difficulties has been proposed (Schmid, 1970, Schmid et al 1971, Schmid 1974, Schmid et al., 1975). This model has also been used for clinical applications (Schmid et al., 1973, Mira et al., 1975) but it is not capable of reproducing the many anomalies of electronystagmographic (ENG) tracings (Mira et al., 1975). For this we decided to elaborate a model in order to overcome this shortcoming. It must be stressed that, for lack of physiological data, the saccadic pathway of this model is planned only on mathematical bases, whereas the path of the slow phase of nystagmic beats is supported by experimental results. As a matter of fact, it has been shown (Shimazu & Precht,

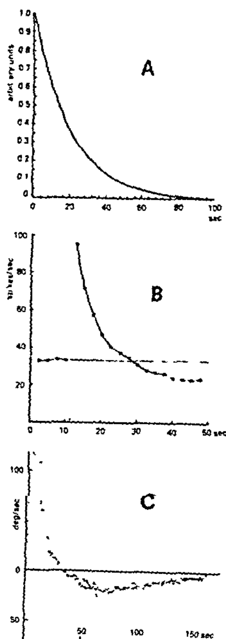


Fig. 1 (A) Theoretical cupular response to a velocity step. (B) corresponding firing rate of some vestibular nuclei units (Shimazu & Precht 1965). (C) slow phase angular velocity of the post rotational nystagmus plotted beat by beat (Malcom & Jones, 1970).

1965) that during postrotational nystagmus some nuclear vestibular neurons exhibit a discharge frequency pattern f_L (Fig 1*B*), which is "copied" by the shape of the eyeball mean velocity θ during the slow phase (Malcom & Jones, 1970) (Fig 1*C*) From this finding it emerges that information represented by cupular position α_c (Fig 1*A*) must be processed in such a way to obtain the shape f_L before reaching the medial longitudinal fasciculus (MLF) Moreover the relationship between θ and f_L appears to be algebraic and the latter must therefore be processed before reaching extraocular muscles (EOM) so as to compensate the characteristics of the eyeball–orbit system, which are not algebraic (Collins, 1971) The experimental finding that the firing rate of oculomotor nuclear neurons increases in a ramp-like manner during every single nystagmic slow phase (Maeda et al , 1971, Baker & Berthoz, 1974) confirms the above hypothesis on the processing of f_L before reaching EOM This will be apparent in the following section

1. Hypotheses on the Mechanism of the Slow Phase of Nystagmus

In this paper the following symbols will be used

t_i' and t_i'' — duration of slow and fast phase, respectively, of the ith nystagmic beat, i=1 , n, n=number of beats

cupular position

firing rate of ampullary neural unities, whose discharge is proportional to α_c (Precht et al , 1971)

frequency modulation of spikes reaching EOM in interval t_i'

eyeball mean angular velocity during the slow phase of the ith beat

slow phase angular velocity of the nystagmus plotted beat by beat against the time after the onset of the stimulus

θ_i — eyeball angular position during the ith beat

It must be pointed out that the dependence of the aforesaid signals on the time or on the frequency domain will be indicated explicitly only when it proves necessary for the sake of comprehension

In order to save the model from useless detail, we have introduced the following hypotheses (which are only approximately exact) During the slow phase (interval t_i')

the contraction strength developed by EOM is proportional to f_{0Li}' (Keller & Robinson, 1972),
corresponding motor torque (C_{mi}) is proportional to f_{0Li}' because the arm of the torque varies in a negligible way when θ varies,
the relatively small value of acceleration $\ddot{\theta}_i$ in interval t_i' allows us to consider the system as lacking inertia in the afore said interval (Keller, 1973)

The nuclear vestibular neurons' response pattern (Fig 1*B*) and the ampullar neural unities' frequency f_c proportional to α_c (Fig 1*A*) allow us to assume that the transfer function (S_i') between the vestibular nuclear neurons' response frequency (f_L) and the ampullar neural unities' firing rate (f_c) must be as follows

$$\frac{f_L}{f_c} = \frac{s}{s+K} \qquad (1\cdot1)$$

where K is a constant ($\approx$(1/80) sec^{-1}) [1]

[1] Such a value depends on the long term constant of semicircular canals (10 sec (Malcom & Jones 1970)) and the instant (usually 35 sec) in which inversion of postrotational nystagmus takes place

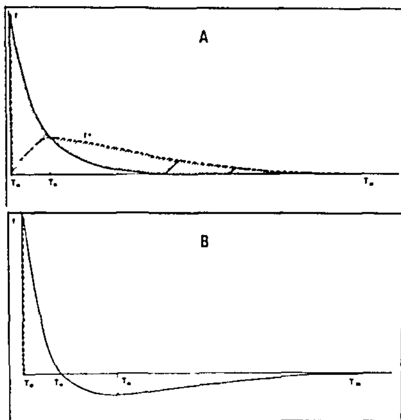


Fig. 2 (*A*) Shape of f_c (solid line) and f^* (hatched line) (*B*) shape of f_L

In fact, note that f_L, schematized in Fig. 2*B*, can be obtained by subtracting the diagram of f^* from that of f_c (Fig. 2*A*). f^* is a signal obtained by sending f_c to the input of a leaky integrator having a time constant of $1/K \approx 80$ sec and a gain K, i e

$$f^* = f_c \frac{K}{s+K} \qquad (1\text{-}2)$$

it follows that

$$f_L = f_c - f_c \frac{K}{s+K} \qquad (1\text{-}3)$$

We may write

$$f_L = f_c \frac{s}{s+K} \qquad (1\text{-}4)$$

Moreover as it has been observed in the Introduction, the shape of θ reproduces, for discrete values, that of f.

It follows that

$$\frac{\theta_l}{f_L} = \text{constant} \qquad (1\text{-}5)$$

during t'_l

On the other hand, the motor torque C_m acting on the eyeball must equal the eyeball–orbit resistant torque, therefore, on the basis of the hypotheses made on eyeball dynamics, we have

$$C_{ml} = N\theta_l + Ds^{-1}\theta_l \qquad (1\text{-}6)$$

but we supposed C_{ml} proportional to f'_{0Ll}, and then

$$\frac{\theta_l}{f'_{0Ll}} = K_Q \frac{s}{s+D/N} \qquad (1\text{-}7)$$

K_Q = constant of proportionality

By comparing equation

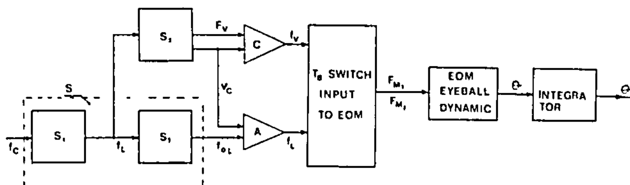


Fig 3 Schematic diagram of the model

emerges that, between vestibular and oculomotor nuclei, f_l must be processed so as

$$\frac{f'_{0L1}}{f_L} = \frac{s+D/N}{s} \tag{1-8}$$

In fact, only in this case can the condition expressed by the eq. (1-5) (based on the quoted findings of Shimazu & Precht, 1965) be verified It is then evident that f'_{0L1} can be obtained by summing to f_l a signal proportional to its integral according to the constant D/N

2. The Model

The model is shown in Fig 3 Subsystems S_1 and S_2 account for the slow and the fast phase of nystagmus, respectively S_1 is split in subsystems S'_1 and S''_1 standing for the transfer functions specified by equations (1-1) and (1-8) The output of S_1 (f'_{0L}) and that of S_2 (F_V) reach EOM through gate A (anticoincidence) and C (coincidence) both driven by a waveform (V_C) with logical levels ZERO and ONE When the stimulation is such that the output of S'_1 (f_L) does not reach a threshold level (F_{LS}) S_2 is off and V_C ZERO In this way, only slowly varying signals can reach EOM On the other hand, for $f_l > f_{LS}$ there is activation of S_2, which acts as a timer switching V_C from ONE to ZERO and vice versa, thus causing the beginning of intervals t_l and t''_l, respectively In fact when V_C is ZERO C is off and A is on, so that f_{0L} reaches EOM thus causing the slow phase when V_C is ONE A is off and C is on so that EOM are reached by F_V and fast phase takes place Switch T_B properly driven, conveys to EOM the outputs of the model with such a phase as to cause clockwise and counterclockwise movements of the eyeball, according to stimulation

Apart from the analog signals v' and v" (Fig 4), the signals flowing in the model are pulses of constant amplitude, which convey information according to the law of temporal variation of their frequency, this information will be considered continuous time function

STRUCTURE AND BEHAVIOUR OF S_1

Our model is represented in a detailed form in Fig 4 [1] S'_1 is implemented by the following components a reversible numerical counter (C_L), a digital analog multiplier (M_L), a switch (T_A) and a frequency adder (Σ_L) The number n_L stored in C_L controls M_L supplied by a con-

[1] To explain more easily the mechanism of vestibular nystagmus nystagmic threshold was not introduced in Fig 4 this shortcoming will be overcome in section 5

Fig 4 Diagram of the model in detailed form

stant frequency (F_0) The output signal f^* of M_L is expressed by the following equation

$$f^* = K\, n_L \qquad (2\ 1)$$

where K is a constant given by the product of F_0 by a constant whose value depends on the particular numerical code used by S_1 The cupular output f_c is applied to the summing input S of C_L The difference input D of the latter is supplied by f^*, therefore

$$n_L = s^{-1}(f_c - f^*) \qquad (2\ 2)$$

If we insert eq (2 1) the n_L value as given by eq (2 2) and we solve for f^*, we may write

$$f^* = f_c \frac{K}{s+K} \qquad (2\text{-}3)$$

f^* is applied to one of the two inputs of the frequency adder Σ_L The other input of Σ_L is supplied by the cupular output f_c f_c and f^* are applied to Σ_L through the two position switch T_A driven so as to be in position 1 for $f_c > f^*$ and in position 2 for $f_c < f^*$ For this (see Fig 4) the frequency of the signal applied to the summing terminal S of Σ_L is always greater than that applied to difference terminal D it follows that the output f_L of Σ_L (Fig 5) is expressed by the equation

$$f_L = |f_c - f^*| \qquad (2\text{-}4)$$

f_L is applied to S_I'' through the anticoincidence gate A_0 driven by V_L Therefore the output f_{0L} of A_0 is identical with f_L or zero in the intervals t_i' or t_i'', respectively Subsystem S_I'' is implemented by the following components a reversible numerical counter (C_I), a digital analog multiplier (M_I) and a frequency adder (Σ_I) f_{0L} is applied to terminal S of C_I, whose stored number drives M_I which in turn is supplied with a constant frequency (F_I) It follows that output f_{0I}^* of M_I is given by

$$f_{0L}^* = s^{-1} K_I\, f_{0L} \qquad (2\text{-}5)$$

K_I = constant of proportionality

f_{0L} and f_{0L}^* are added in the frequency adder Σ_I whose output f_{0L}' is therefore related to f_{0L} by the transfer function

$$\frac{f_{0L}'}{f_{0L}} = \frac{s+K_I}{s} \qquad (2\ 6)$$

For what we have said in the previous section, S_I'' must be so designed that

$\cdot_I = D/N$

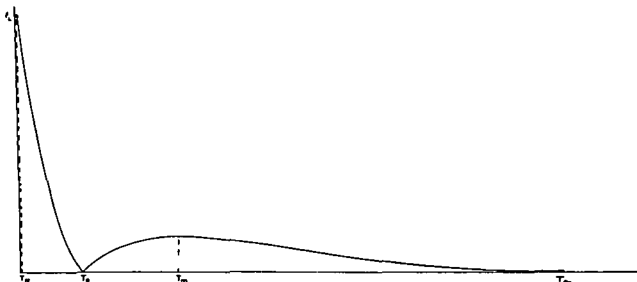


Fig 5 Shape of f_L in the model

Note that terminal D of C_I is supplied, only in the time intervals t_i'' in which A_0 is off, by a signal delivered by subsystem S_2 which will be discussed in the following part of this section

STRUCTURE AND BEHAVIOUR OF S_2

Subsystem S_2 is composed of the following components

Pulse rate meter M f_L is applied to its input, therefore M delivers the analog signal v' given by the equation

$$v'=K_M f_L \quad (2\ 8)$$

K_M=constant of proportionality
It follows from (2-4)

$$v'=K_M|f_c-f^*| \quad (2\ 9)$$

Therefore v is not negative and becomes zero only for $f_c=f^*$

Reversible counter C_V C_V is coupled to the digital analog converter N whose analog output v" is

$$v''=K_N n_V \quad (2\ 10)$$

K_N=constant of proportionality
n_V=number stored in C_V

As will be shown further on, v" ranges cyclically from zero to v', limits included

Comparator Q v' and v" are applied to its inputs It is so designed that its output V_C is zero (logical level ZERO) when v" is increasing from zero to v' and V (logical level ONE) when v" is decreasing from v' to zero, it switches (in a negligible time) from ZERO to ONE and vice versa when v" equals v' or becomes zero, respectively

Generator F_V It delivers the high constant frequency F_V It is coupled, through a coincidence gate C, to the output of the model and to terminal D of C_V and C_I

Frequency duplicator D The role of this component will be apparent in the following section Note that this component would be unnecessary in a bilateral model

The output V_C of Q drives both coincidence gate C and anticoincidence gates A_0 and A For this, when V_C is ZERO, A_0 and A are on C is off and therefore only terminal S of C_V is supplied and v" increases With this statement and remembering that v' is not negative (2 9), the behaviour of S_2 may be so summarized let t=0 be a generic instant in which v"=0 (C_V reset) in t=0 V_C is ZERO, only the terminal S of C_V is supplied therefore v" increases this phase ends in the instant t=t,

in which v'' equals v' and causes the switching of V_C to ONE. From then on, only the terminal D of C_V is supplied, therefore v'' decreases (rapidly), owing to the high frequency of F_V). this phase ends in the instant $t=t'+t''$ in which v'' becomes zero, in this instant, in fact, V_C again switches to ZERO, re-establishing the situation which existed when $t=0$, thus causing both the end of the cycle under consideration and the beginning of the following one. The waveform of v', v'' and V_C in generic intervals t_i-1, t_i and t_i+1 are represented in Fig. 6. The intervals t' and t'' of the ith beat have already been called t'_i and t''_i, where, obviously,

$$t'_i+t''_i=t_i \tag{2-12}$$

As can be observed in Fig. 4, f_L and $2f_V$ are the two outputs of the model, from the previous considerations, it appears that in the intervals $t''_i f_L$ is zero and $2f_V$ is equal to $2f_V$, moreover f_L and $2f_V$ are the signals which will cause the slow and fast movements, respectively, of the eyeball.

Switch T_B. The two outputs f_L and $2f_V$ of S_2 are applied to the terminals U' and U'' through the two-way and two-position switch T_B, which is driven synchronously with T_A. It is therefore switched to position 1 when $f_c>f^*$ and to position 2 when $f_c<f^*$, it follows that, when $f_c>f^*$, the slow and fast signals relative to each cycle are applied to the terminals U' and U'' of the model, respectively, whereas the opposite happens when $f_c<f^*$

3. Transfer of the Signals Delivered by the Model to the EOM

A diagram of a possible connection between the terminals U' and U'' of the model and the EOM is reported in Fig. 7, where it may be seen that $F_B(F_B \ll F_V)$ is a tonic activity, the components $\Sigma_i(i=1, \ldots, 4)$ are frequency adders, in which the output frequency f_i is given by the equation

$$0\leq f_i\leq 2F_V \tag{3-1}$$

$2F_V$ being the frequency of the pulses delivered by the model in the intervals t''_i. Therefore, whichever negative value the algebraic sum of the frequencies applied to the two terminals of the adder takes on in a generic time interval, in that interval f_i will be zero. Instead, if this sum is greater than $2F_V$, $f_i=2F_V$. C' and C'' are coincidence gates, A' and A'' are anti-coincidence gates. These circuits are controlled by the output V_C of the comparator Q (Fig. 4) either directly (switch T_C in position 1) or through the inverter I (switch T_B in position 2). Furthermore, it must be noted that T_C is driven synchronously with T_A and T_B, therefore it will be in position 1 when $f_c>f^*$ and in position 2 when $f_c<f^*$. The output signal f_i of the circuits Σ_i are applied as indicated in Fig. 7 to the circuits OR_1 and OR_2, whose outputs (signals with frequency f_{M1} and f_{M2}, respectively) directly stimulate the EOM M_1 and M_2, according to Fig. 7, the eyeball will move clockwise or counterclockwise when $f_{M1}-f_{M2}$ is positive or negative, respectively. Bearing these preliminary considerations in mind, the behaviour of the circuitry represented in Fig. 7 may be so described.

(*a*) In the steady state (i.e. when f_c is either zero or constant, independent of the state of V_C and of the position of T_C) one has

$$f_{M1}-f_{M2}=F_B \tag{3-2}$$

in these conditions, M_1 and M_2 are stimulated by the same tonic activity F_B, therefore the eyeball does not move. When f_c varies, the situation changes according to whether T_C is in position 1 or 2.

(*b*) T_C switched in position 1 ($f_c>f^*$) during the interval t'_i, terminal U' is supplied by

Fig 6 Waveform of v , v″ and V_C during intervals t_i-1 t_i and t_i+1

V_C is in state 0, therefore gates A′ and A″ are on, consequently the situation is as follows

$F_B+f'_L$
$f_4=0$
$f_3=F_B-f'_L$

Therefore,
$f_{M1}-f_{M2}=2f'_L$ (slow clockwise movement)

During interval t''_i $2f'_V$ reaches terminal U″, V_C is in STATE 1, therefore the on gates are C′ and C″, this means that

$f_1=f_3=0$
$f_4=F_B+2f'_V$
$f_2=F_B-2f'_V$

considering that, in the t''_i interval, f'_V equals F_V, which is much greater than F_B, and also bearing in mind eq (3 1) it then follows that

$f_4=2f'_V$
$f_2=0$

$f_{M1}-f_{M2}=f_2-f_4=-2f'_V$ (fast counterclockwise movement)

(*c*) T_C switched in position 2 ($f_c<f^*$) during interval t'_i, U″ is supplied by f'_L, V_C is in STATE 0, therefore (as the inverter is on) C′ and C″ are on and

$f_1=f_3=0$
$f_2=F_B-f'_L$
$f_4=F_B+f'_L$

$f_{M1}-f_{M2}=f_2-f_4=-2f'_L$ (slow counterclockwise movement)

During interval t''_i, terminal U′ is supplied by $2f'_V$, V_C is in STATE 1, therefore (as the inverter is on) A′ and A″ are on and

$f_2=f_4=0$
$f_1=F_B+2f'_V$
$f_3=F_B-2f'_V$

actually, for the above-mentioned reasons,

$f_1=2f'_V$
$f_3=0$,

which to conclude gives us

$f_{M1}-f_{M2}=f_1-f_3=2f'_V$ (fast clockwise movement)

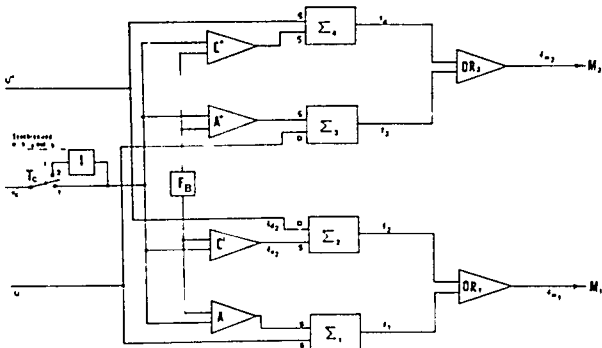


Fig 7 Possible diagram of the transfer to EOM of the signals delivered by the model

According to the structure of the model the strength of θ_i (interval t_i) is nearly proportional to f_L (which is constant in t_i) Instead in interval t_i'' the aforesaid velocity is nearly proportional to F_V [1]

4. Rotational Stimulation: Behaviour of the Model

A POST ROTATIONAL NYSTAGMUS

As has been stressed the shape of θ reproducing the waveform of f_L corresponds to that found in the physical system (Malcom & Jones, 1970) Moreover $t_i (i=1, \ldots n$ n=number of the beats) is constant (irrespective of i), this is because in t_i' (see Fig 4) both v' and the slope of v" are proportional to f_L, it follows also that the amplitude A_i of the ith nystagmic beat is proportional to θ_i this agrees with Mira et al (1973) for $\theta_i > 3°$/sec

The velocity of the fast phase is the same, irrespective of i, because in interval t_i'' the model delivers the constant frequency F_V it follows also that t_i'' is proportional to A_i Moreover $t_i (= t_i' + t_i'')$ is nearly constant, irrespective of i, because $t_i'' \ll t_i'$ For a sufficiently strong nystagmus this agrees with several experimental findings (Mira et al , 1973, Cheng & Outerbridge, 1974)

Inversion of nystagmus is also reproduced by the model because when $t > T_0$ (see Fig 2*A*) $f_c < f^*$ and T_B is switched in position 2,

[1] As far as t_i is concerned this follows from the assumption made in section 1 (response of EOM to stimulation and dynamic behaviour of EOM-eyeball) and from the structure of S* (which implements the transfer function of eq (1-8)) Instead in interval t'' we cannot neglect the inertia of the eyeball but we must note that in the aforesaid interval eyeballs are driven both by the torque developed by F_V and the elastic torque of orbital tissue (note that in interval t_i'' gate A is off see Fig 4) Moreover both elastic and inertial torque are decreasing with decreasing θ so that we may assume that they compensate each other

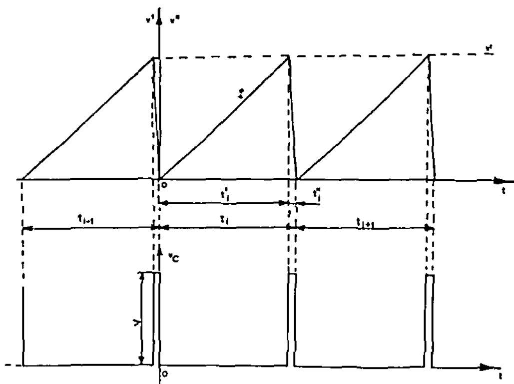


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Therefore,
$f_{M1}-f_{M2}=2f'_L$ (slow clockwise movement)

During interval t''_i, $2f'_V$ reaches terminal U", V_C is in STATE 1, therefore the on gates are C' and C", this means that

$f_1=f_3=0$
$f_4=F_B+2f'_V$
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considering that, in the t''_i interval, f'_V equals F_V, *which is much* greater than F_B, and also bearing in mind eq (3 1), it then follows that

$f_4=2f'_V$
$f_2=0$

$f_{M1}-f_{M2}=f_2-f_4=-2f'_V$ (fast counterclockwise movement)

(*c*) T_C switched in position 2 ($f_c<f^*$) during interval t'_i, U" is supplied by f'_L, V_C is in STATE 0, therefore (as the inverter is on) C' and C" are on and

$f_1=f_3=0$
$f_2=F_B-f'_L$
$f_4=F_B+f'_L$

$f_{M1}-f_{M2}=f_2-f_4=-2f'_L$ (slow counterclockwise *movement*)

During interval t''_i, terminal U' is supplied by $2f'_V$, V_C is in STATE 1, therefore (as the inverter is on) A' and A" are on and

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$f_1=F_B+2f'_V$
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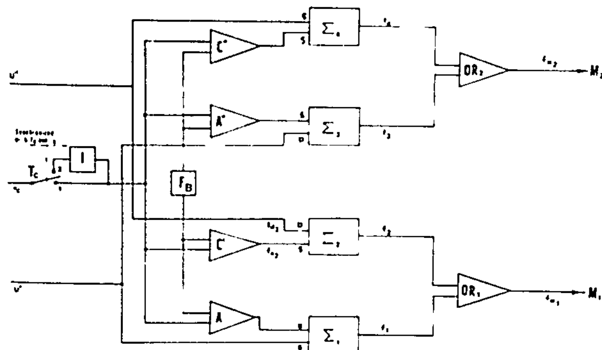


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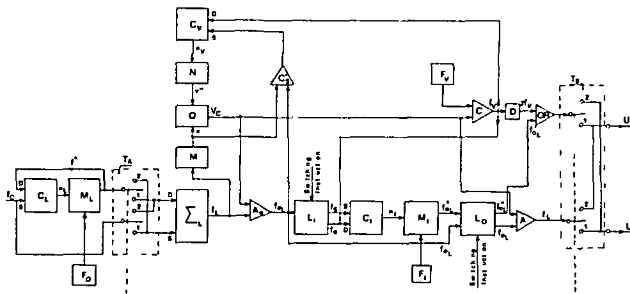


Fig 8 Diagram of the model inclusive of the circuit accounting for nystagmic threshold

this means that for $t>T_0$ the eye movement direction both during the slow and the fast phase, will be opposite to that resulting for $t<T_0$

By choosing F_0 conveniently, it is possible to approximate to the wanted precision degree the instant T_0, in which f_c and f^* intersect, to the experimentally found one

Note that between primary and secondary nystagmus the value of f_L is subthreshold, this accounts for the lack of beats around T_0 In every beat the model delivers the same number of pulses both in slow and fast phase, moreover the last nystagmic movement will always be a saccade

Lastly we want to stress that during each *saccadic movement the synergistic is stimulated* by the very high frequency $2f'_V$ (see Fig 7), whereas the antagonistic muscle is not stimulated (Bjork & Kugelberg, 1953, Miller 1958, Tamler et al 1959 Schaefer, 1965)

B CONSTANT ANGULAR ACCELERATION

It is well known that if an individual is placed on a rotating chair moving with a constant angular acceleration, the nystagmic response, maximal at the beginning, attenuates until it disappears This phenomenon of adaptation is reproduced by the model As a matter of fact, under such a stimulation, cupular output f_c becomes constant in the steady state and it will be

$$f^*=f_c$$
$$f_L=0$$
$$f'_L=f'_V=0$$

C SINUSOIDAL STIMULATION

Up to this point, it has been implicitly considered that the cupula in the steady state and with angular stimulation equal *to zero delivers* no pulse, but this is not so, because even in the above-described conditions the cupula exhibits some tonic activity and delivers pulses with constant frequency Γ_R, this, however, is not in contradiction to our previous statements because the cupular background activity (as can be easily verified) adds up to the signals applied to both the terminals of Σ_L (Fig 4) and is in this way eliminated In sinusoidal stimulation thanks to Γ_R, the fre

quency of the cupular signal does not become negative.[1] From these considerations and from what has been said on the model behaviour, one may easily deduce that during sinusoidal stimulation f_L consists of a full wave sinusoidal rectified signal. Since f_L is processed in the same way as the post-rotational signal, we may conclude that the model is able to reproduce sinusoidal stimulation, too.

5. Modifications to Extend the Validity of the Model to the Case of Stimulations below the Nystagmic Threshold

The proposed model delivers signals which have the characteristics described in section 2 (i.e. they are composed of successions of cycles of generic duration t_i) irrespective of the strength of $f_c(t)$, i.e. of the head movement which delivered it. This prevents the use of the model in some very important cases, such as those related to the slow eyeball movements in response to head movements which produce accelerations below the nystagmic threshold. However, this drawback may be easily overcome by slightly modifying the structure of the model, as indicated in Fig. 8. In this latter, f_{0L} is delivered to terminal S of C_5 not directly, as in Fig. 4, but through gate C'_5 driven by the output v' of M. C_5 is so planned as to be on only when v is greater than an established threshold V'_5. In this way, when stimulation is such that $v < V_5$ subsystem S_2 is no longer operative. The presence, in the model, of set C_I–M_I (Fig. 8) creates some problems as far as the logic of the output and input of the set itself is concerned. In fact, note that if subsystem S_2 is not operative, A_0 and A must always be on and C always off. Moreover, because signal $s^{-1}K_I f_{0L}$ delivered by M_I must compensate the eyeball elastic torque, number n_I stored in C_I must increase in time interval t_a and decrease in interval t_b, being t_a and t_b intervals in which the eyeball moves away from or returns to reference position, respectively. The need to add output of M_I to f_{0L} in interval t_a and to subtract it in interval t_b is obvious too. For the sake of brevity, the problem just mentioned will not be discussed here, but in a succeeding paper.

We wish merely to stress that the aforesaid model has been simulated on a numerical computer; actually we are identifying the same performing experimental trials by a computer-driven rotating chair, Polman Digital 1S, coupled to an electronystagmograph, Polman RM 69.

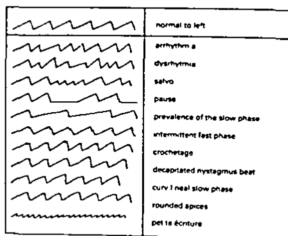


Fig. 9. Main ENG anomalies (revised form by Cocchini & Dufour 1975).

[1] Naturally this is not true when sinusoidal signal amplitude is greater than tonic frequency F_R, but we believe that such a shortcoming may be overcome considering the interaction between the two labyrinths.

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A histological, histochemical and ultrastructural study

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A histological, histochemical and ultrastructural study

BY

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OULU 1977

Kirjapaino Osakeyhtiö Kaleva
Oulu, Finland 1977

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I INTRODUCTION

A Experimental respiratory tumor induction

Evidence for a relationship between exposure to environmental carcinogens and the increasing lung cancer incidence depends primarily on epidemiological data The difficulties in experimental evaluation of suspected carcinogens relate to the fact that the type of lung cancer generally seen in man has rarely been observed in experimental animals Most experimental work in this area has depended upon tumor induction in animals in tissue other than that of the respiratory system or of lung tumors of substantially different types than those in man

In early studies systemic application of carcinogens showed an increased frequency of spontaneous adenomas in mice (Andervont 1937) These tumors have been shown to be due to proliferation of type 2 granular lung pneumocytes (Brooks 1963) The lung adenomas are markedly dissimilar from those of man in that they are multiple (up to 50 tumors per animal have been observed) almost invariably benign slow growing and kill the host only by replacing normal respiratory tissue

Another method effective in producing a marked respiratory tumor response involves oral intraperitoneal or subcutaneous application of nitrosamines Several of these compounds notably diethylnitrosamine have been shown to be effective (Herrold 1970 Montesano *et al* 1971 Harris *et al* 1974) However such compounds mainly induce tracheal papillomas and polyps and adenosquamous peripheral lesions the latter rarely seen in man (Stenbäck 1974) Also this method of exposure i e systemic subcutaneous and intravenous injections is artificial compared to the human situation

Implantation of carcinogenic material into the chest cavity has been used since Andervont (1937) induced adenomas adenocarcinomas and squamous cell carcinomas in mouse lung by this method (Stevenson and von Haam 1963) Kuschner *et al* in a series of studies (Kuschner 1968) showed the efficacy of this method by implanting benzo(a)pyrene (B(a)P) methylcholanthrene (MC) and such radioactive compounds as P^{32} and producing adenocarcinomas and squamous cell carcinomas Pellets introduced by thoracotomy into the rat chest cavity have been used (Stanton & Blackwell 1961 Stanton *et al* 1972 Kinosita 1966) for evaluating the carcinogenicity of various compounds in inducing squamous cell carcinomas in the rat These methods were effective However the nonspecific injury associated with this technique as well as the lack of relation to the human situation decreases its applicability for respiratory tumor induction

Induction by inhalation exposure to carcinogenic compounds is highly comparable to the human situation Animals are exposed to the test substance in a chamber where the mixture of air and chemical humidity temperature etc is carefully controlled This method however requires complicated equipment expertise in handling and conducting the studies and is

costly The resulting tumor incidence is also low and the induction period long—several months up to 2—3 years (Kuschner *et al*, 1975)

A technique which combines several advantages of the previously mentioned methods is intratracheal instillation of polycyclic hydrocarbons Early studies were basically negative, i e, either no tumors resulted (Burrows & Boyland, 1938) or only a very few peritracheal sarcomas (Sannie *et al*, 1935) In 1949, Niskanen was able to induce squamous cell carcinomas in 6 of 25 rats with repeated intratracheal injection of a suspension of dibenz(a,h) anthracene in olive oil Also Della Porta induced squamous cell carcinomas of the larynx and trachea in Syrian golden hamsters by administering a colloidal suspension of 7,12 dimethylbenz(a) anthracene (DMBA) into the trachea (Della Porta *et al*, 1958) Pylev (Pylev, 1961, 1963, 1964) and Shabad (Shabad 1962) induced bronchiogenic carcinomas in rats by intrabronchial injection of carcinogenic polycyclic hydrocarbons dispersed in black ink powder Herrold and Dunham (1962) administered a suspension of 3 4 B(a)P in Tween 60 by intracheal instillation and urethan by subcutaneous ction to Syrian golden hamsters and succeed in inducing a high yield of respiratory tract tumors including bronchiogenic carcinomas The efficacy of this technique was subsequently confirmed (Miller *et al* 1965) Another useful technique for respiratory tumor induction employed carcinogenic polynuclear hydrocarbons prepared as suspensions of fine crystalline particles attached to a fully particulated inert dust suspended in saline and administered intratracheally (Saffiotti *et al* 1968 1972) The morphology of these tumors was very close to that in humans squamous cell carcinomas were the most frequent type followed by anaplastic carcinomas and adenocarcinomas

B. Morphological aspects

Interest in definition of specific preneoplastic changes is twofold determination of stages of development of tumors relating to cell type of origin giving us an understanding of the stages and morphogenesis of the tumor type as well as determination of cellular and tissue changes of prognostic significance indicating later manifestation of neoplasms The significance of preneoplastic alterations have been amply demonstrated for cancer of the cervix in man as well as in experimental animals (Scarpelli & von Haam, 1960, Stenbäck, 1970) and in skin (Stenbäck, 1969) Studies on respiratory tumor formation have been less successful in this respect (Nasiell, 1968) Sputum cytology is, however, a commonly accepted method for detection of lung cancer, though less is known about specific preneoplastic conditions in the respiratory tract of man (Papanicolau, 1956, 1958)

Preneoplastic conditions claimed to have biological significance in the lung include squamous metaplasia in man (Auerbach *et al* 1956, 1957a 1957b) and experimental animals (Saffiotti, 1968, Harris *et al* 1971), ciliary alterations quantitative and qualitative (Nasiell, 1963, Port *et al* 1973), as well as changes in the basal membrane and nuclear and nucleolar structure (Harris *et al*, 1971) Alterations associated with neoplastic progression in carcinogen treated skin also include histochemically detectable changes in cell maturation and differentiation keratin formation and mucin formation, as well as activity and distribution of enzymes relating to cellular activity and metabolism (Stenbäck, 1969)

Squamous cell carcinomas are the most common of all types of human lung tumors The incidence of this neoplasm has shown the greatest increase among all tumor types in recent years and is most clearly associated with cigarette smoking (Kreyberg 1962 1967, Wynder *et al* 1970) Previous studies have shown the significance of tumor cell type in terms of biological behavior (Haupt & Fuchs 1966 Larson 1973), on the other hand some authors consider histological classification of lung cancer arbitrary and have questioned its value (Willis 1966 Salzer, 1971, Wildner, 1966 Matthes *et al* 1969) The reliability and consistency in classifying lung neoplasms has also been questioned (Herrold 1972) probably as a con

sequence of a lack of uniform criteria and differences in morphological classification

In man lung adenocarcinoma is less common than squamous cell carcinoma, but has increased in frequency in relation to cigarette smoking (Haenzel *et al* 1962 Hammond, 1966) and occupational exposure (Archer *et al*, 1974) The exact incidence of this tumor type, its formation, morphology and significance for prognosis have not been completely settled, partly because of the somewhat ambiguous criteria applied to its morphology Kreyberg (1967) presented two main subgroups of this tumor type bronchiogenic acinar, and papillary and bronchioloalveolar, Matthews, 4 groups well, poorly and moderately differentiated and bronchioloalveolar/papillary adenocarcinomas (Matthews, 1973) and Green *et al*, 2 groups bronchiolar carcinomas and adenocarcinomas (Green *et al*, 1972) Others have avoided sub classification (Shinton, 1962, Campobasso, 1968, Walter & Pryce, 1955) The possibility that this tumor may actually be formed of different cell types may account for the variety of published reports of incidence, biological behavior and prognosis (Doll *et al*, 1957, Green *et al*, 1971, Spain, 1959, Weiss *et al*, 1970)

Tracheal carcinomas in humans are rare, and, thus far only about 500 cases have been reported (Dalby & Jones, 1961, Frable & Wheelock, 1962, Huguenin Dumittan *et al*, 1966, Mc Cafferty *et al*, 1964, Hajdu *et al*, 1970) Of 1,000 patients with malignant respiratory system tumors, one neoplasm can be expected to be a primary tracheal carcinoma (Moerch *et al*, 1954, Acquarelli *et al*, 1967, Houston *et al*, 1969) Burn (1957) reports 2 tracheal tumors out of 29 000 autopsies and Culp (1938), 7 tracheal tumors in 97,335 autopsies It is not clear why malignant tracheal neoplasms are rare, while bronchial and laryngeal carcinomas are fairly common, despite the fact that these structures are continuous and histologically similar (Ranke *et al*, 1962, Acquarelli *et al*, 1967)

Intratracheal instillation of Syrian golden hamsters with certain polycyclic hydrocarbons has been effective in producing a number of tracheal tumors (Stenbäck, 1973) The compounds used have included common environmental contaminants, the polycyclic hydrocarbons, such as 3,4 benzo(a)pyrene (B(a)P), dibenz(c,g)carbazole (DBC) or dibenz(a,i)pyrene (DBP) alone or in combination with different dusts, including talc, titanium oxide (TiO_2) or ferric oxide (Fe_2O_3) (Stenbäck, 1974, Stenbäck and Sellakumar, 1974, Stenbäck *et al*, 1975)

C Tumor classification

The most widely used system for classification of tumors of the respiratory tract is the WHO nomenclature based mainly on Kreyberg's studies (Kreyberg, 1962, 1967) Many studies use different classifications basically similar to Kreyberg's, but a smaller number of groups (Walter & Pryce, 1955, Shinton, 1962, Campobasso, 1962) or for other reasons, different as previously mentioned One of the objections has been that the WHO classification is mainly descriptive, not taking into account histogenetical or biological aspects This classification has not been used in experimental studies to a major extent and its applicability to this type of study or relationship to biological aspects of tumor behavior is less known

II PURPOSE

In this study we will attempt to describe morphological changes induced by i t instillation of carcinogens and dust in the hamster lung, metaplastic, dysplastic and premalignant alterations and the different types of respiratory tumors formed, as observed by histological, histochemical and ultrastructural, transmission electronmicroscopic and scanning electron microscopic methods

The purpose is to

1. To study morphological alterations during tumor induction by different agents and their specificity and significance in relation to ultimate formation of malignant tumors
2. Study the various morphological and biological characteristics of the different types of tumors observed and attempt to relate tumor structure to cell type of origin, mode of progression and biological behaviour of the tumor as well as tumor location trachea, bronchi, bronchioli or alveoli, in comparison to man
3. Attempt to devise a classification for these tumors and compare with the WHO classification to determine its relationship to histogenetic aspects and suitability for experimental studies

The purpose of this study is to gain understanding in the causation and behavior of respiratory system tumors useful in studies on detection, prevention, treatment and prognosis of these neoplasms

III MATERIAL AND METHODS

A Tumor induction

The experimental material came from 1600 randomly bred hamsters of the Eppley Colony The animals belonged to four main groups, 450 animals received the carcinogen alone, 450 animals the dust alone, 450 animals carcinogen and dust and 250 animals received no treatment The animals were kept in plastic cages 4—6 in each and given pelleted diet and water ad libitum

The chemicals 3,4 benzo(a)pyrene (B(a)P) 9 10 dimethylbenz(a)anthracene (DMBA), 7H dibenzo(c,g)carbazole (DBC) or dibenz(a,i) pyrene (DBP) were administered once a week, alone, in combination with ferric oxide (Fe_2O_3) or titanium oxide (TiO_2), magnesium oxide (MgO_2) or talc, or the dust alone, as a saline suspension

The chemicals 0 2 cc of the suspension was given intratracheally (i t) 10—15 times by the method of Saffiotti *et al* (1968) Before treat ment animals were anesthesized with 0 4 ml of a 1 % solution of sodium brevital, (sodium α-dl 1 methyl 5 allyl 5-(1 methyl 2 pentynyl) bar biturate) The animals were killed during the experiment or allowed to die spontaneously All animals were checked regularly At autopsy, lungs were excised fully expanded, as was the trachea including mediastinal organs The entire specimen was fixed in 10 % buffered formalin and subsequently sections were taken from all lung lobes and the trachea

B Morphological methods

Formalin fixed specimens were embedded in paraffinsections 5 μ thick were cut at 3—5 levels or more if necessary Hematoxylin eosin stain was used

For differentiation of fibrous components Masson's, Snook's, Verhoeff's and Gomori's stains were used Keratinisation was studied by Kreyberg s, ninhydrin, gram and methylgreen pyronin stains For differentiation of neutral mucosubstances and glycogen, periodic acid Schiff (PAS) with and without diastase diges tion was used, for acid and sulphonated muco-substances, Alcian blue at pH 0 4 and 2 5 with and without hyaluronidase extraction and Azure A at pH 0 5, 2 0, 3 0 and 5 0 and $MgCl_2$ at concentrations of 0 1, 0 4, 1 0 and 1 5 Sections were also immediately immersed in liquid nitrogen and the enzyme activity of six hydrolytic enzymes and three oxidative enzymes was determined (Pearse, 1974 Stenbäck, 1969) These sections were compared to results from studies using human lung tissue

In selected animals, 1 ml glutaraldehyde (2 5 % in Sorensen's buffer) was injected i t and, after the chest walls were opened, 1 ml glutaraldehyde was injected intracardially After this prefixation, the trachea, with larynx and stem bronchi attached, was removed The tissues from these animals were divided into 1 x 3 mm pieces and postfixed in 1 % buffered osmium tetroxide for 1 hour. They were

II PURPOSE

In this study we will attempt to describe morphological changes induced by i t instillation of carcinogens and dust in the hamster lung, metaplastic dysplastic and premalignant alterations and the different types of respiratory tumors formed, as observed by histological, histochemical and ultrastructural, transmission electronmicroscopic and scanning electron microscopic methods

The purpose is to

1. To study morphological alterations during tumor induction by different agents and their specificity and significance in relation to ultimate formation of malignant tumors
2. Study the various morphological and biological characteristics of the different types of tumors observed and attempt to relate tumor structure to cell type of origin mode of progression and biological behaviour of the tumor as well as tumor location trachea, bronchi bronchioli or alveoli in comparison to man
3. Attempt to devise a classification for these tumors and compare with the WHO classification to determine its relationship to histogenetic aspects and suitability for experimental studies

The purpose of this study is to gain understanding in the causation and behavior of respiratory system tumors useful in studies on detection, prevention treatment and prognosis of these neoplasms

IV. RESULTS

A. Trachea

1 Normal epithelium

The surface epithelium of the tracheal as well as bronchial mucosa of untreated hamsters consists of ciliated mucous and basal cells Beneath the basal membrane are the supporting structures, collagen and elastic fibrils, cartilage rings in the trachea and sparse mucous glands Basal cells form a single cell layer closest to the basal membrane They are fairly small uniform, polygonal cells with a prominent, oval, centrally located nucleus and a small nucleolus Basal cells are attached to the basement membrane by hemidesmosomes and to adjacent cells by des mosomes The intercellular space is narrow, and cytoplasmic interdigitations are present Free ribosomes filaments, and rarely Golgi com plexes are observed in the cytoplasm Mucous cells are less frequent than in other species including man Mucous stains reveal a con tinuous alcianophilic layer on the surface, as well as in droplets in the cytoplasm of mucous cells Large packets of mucous granules are observed at the cell apex and stacks of rough endoplasmic reticulum are located primarily in the basal area Mitochondria are oval or elongated and contain typical horizontal cristae Ciliated cells comprise about 50 % of the total population as determined by SEM The cilia are erect and free standing with filiform projections or microvilli between them The ciliated cells were attached to adjacent columnar cells by desmosomes and by tight junctions at the luminal surface A narrow intercellular space separates the cells At the cell apex are typical cilia rooted in the cytoplasm by basal bodies In addition narrow cytoplasmic processes project into the luminal surface The nucleus is some what irregular in contour and contains a small, round nucleolus with a nucleolonema Numerous mitochondria, free and membrane bound ribosomes and a few filaments are found in the cytoplasm

2 Nonspecific epithelial alterations

These were detected within hours after the first instillation of carcinogen and mainly related to the toxic properties of the treatment The alterations consisted of ciliacytophtoria, lack of cilia epithelial disorganization and cellular enlargement (Fig 1) of focal epithelial des quamation and slit formation was occasionally observed (Fig 2) Micropapillomatosis, small stromal exteriors covered by regular epithelium (Fig 3), as well as lack of cilia (Fig 4) were found

Within a few days after the start of treatment, SEM analysis revealed a denuded area devoid of normal ciliary structure (Fig 5) In other areas short microvilli and tall cilia were preserved (Fig 6) Thin sections studied under SEM showed that microvilli were partly deformed. On the surface, abnormal and aberrant cilia occurred, as well as compound cilia with multiple axial filaments (Fig 7) The remaining

ciliated cells showed swollen, club shaped cilia without central filaments (Fig 8)

Fragmentation and dissolution of columnar cells at different stages, lack of cilia and nuclear plumping and hyperchromasia (Fig 9) become more conspicuous during later stages of the experiment Basal cell hyperplasia with nuclear enlargement and hyperchromasia was observed early (Fig 10) The early destructive changes caused by the continued instillation of car cinogens into Syrian golden hamsters caused widespread disorders in the respiratory tract, resulting in focal areas of squamous metaplasia in tracheobronchial epithelium (Fig 11) The metaplastic areas consisted of layers of large polygonal cells supported by a layer of polyhedral cells of varying thicknesses which showed increased amounts of RNA, -SS and SH groups The cells presented evidence of squa mous metaplasia, with several layers of regularly stratified intercellular bridges and keratinization (Fig 12) Increased amounts of pyroninophilic material in the cytoplasm, as well as the PAS positive diastase reactive material, occurred Transitional type metaplasia, consisted of round cuboid or polygonal cells, and some times showed layers of flattened cells on the surface (Fig 13)

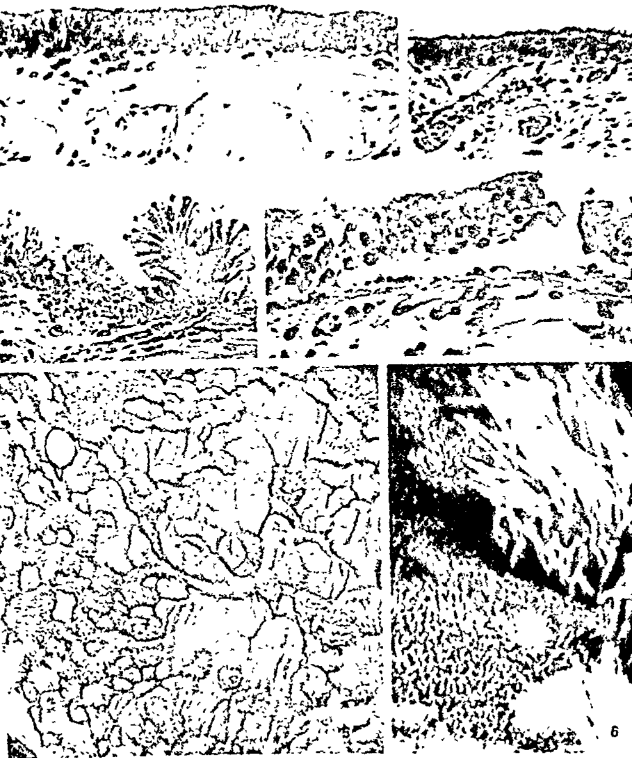


Fig 1 Epithelial disorganization in carcinogen-treated tracheal epithelium (H—E x 262).
Fig. 2. Nuclear enlargement and lack of cilia in carcinogen-treated tracheal epithelium (H—E x 240).
Fig 3 Micropapillomatosis with numerous ciliated and goblet cells in carcinogen-treated tracheal epithelium (Toluidine blue x 300)
Fig 4 Slit formation with early epithelial detachment in carcinogen-treated tracheal epithelium (H—E x 450)
Fig 5 Metaplastic areas of carcinogen-treated tracheal epithelium of Syrian golden hamster. Note areas [illegible]

[illegible] tracheal mu

3 Hyperplastic and dysplastic changes

Continued treatment caused an increase in number and size of epithelial cells, with disturbed polarization and stratification (Fig 14) Squamous metaplastic alterations became more widespread, the squamous cells increased in number and reached the submucous glands (Fig 15) The cells, basal cells in particular, became gradually irregular in size and shape (Fig 16) Lack of cellular differentiation occurred up to the surface, with all cells being of an intermediate cell type Separation into ciliated and mucous cells was difficult (Fig 17) The dysplastic changes could be divided into 3 stages — (1) slight change, with only the basal layer affected, (2) moderate with flattened regular cell on the surface, and (3) severe, affecting all cell layers Ultrastructural analysis showed polymorphic cells with large, hyper chromatic indented nuclei and widened intercellular spaces (Fig 18)

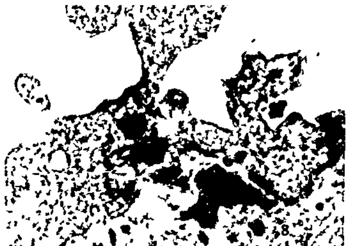
A peculiar feature of carcinogen treated tracheal epithelium was glandular epithelial

Table I *Incidence of Tracheal Epithelial Lesions in Relation to Inducing Agent*

Type of alteration	Treatment		
	None	Dust	Carcinogen
Desquamation	+[a]	++	++
Slit formation	+	++	++
Ciliacytophtoria	+	++	++
Micropapillomatosis	+	++	++
Abnormal cilia	+	+	+++
Basal cell hyperplasia	+	+	+++
Squamous metaplasia	+	+	+++
Transitional metaplasia	+—	+	++
Squamous hyperplasia	—	+	++
Glandular hyperplasia	—	—	+
Epithelial dysplasia slight	—	+—	++
» » , moderate	—	—	+
» » , severe	—	—	+
Basal membrane disintegration	—	—	+

[a]*Occurrence*
— = non existent
+— = very rare
+ = infrequent
++ = regular
+++ = very common

Fig 7 Branched filiform cilia in carcinogen treated tracheal epithelium (Uranylacetate lead citrate x 12 000)
Fig 8 Club-shaped structure without filaments in carcinogen treated tracheal epithelium (Uranylacetate-lead citrate x 28 000)
Fig 9 Epithelial hyperplasia increased number of regularly cells in carcinogen treated tracheal epithelium (Toluidine blue x 480)
Fig 10 Basal cell hyperplasia with preserved cytological structures in carcinogen treated tracheal epithelium (Toluidine blue x 180)
Fig 11 Areas of metaplastic cells with intercellular bridges among well preserved ciliated cells in carcinogen treated tracheal epithelium (Thiocarbohydrazide x 65)
Fig 12 Squamous metaplasia in carcinogen treated tracheal epithelium with keratohyalin granules and preserved desmosomes (Uranylacetate lead citrate x 24 000)
Fig 13 Transitional cell metaplasia in Syrian golden hamster tracheal epithelium induced by carcinogen treatment, showing multiple layers of elongated cells (H—E x 450)








ires 7—13

alterations (Fig 19) The cells were piled up in several layers, with small hollow cyst like structures in the epithelium Prolonged treatment caused formation of intraepithelial gland like structures (Fig 20), a not very common occurrence

The surface epithelial cells showed more frequently a lack of cilia, as well as regenerative and degenerative alterations The cilia were either fingerlike (Fig 21), or presented club formed cilia (Fig 22), deranged filaments (Fig 23) or balloon degeneration (Fig 24)

The incidence of tracheal epithelial lesions in relation to inducing agent is shown in Table 1, indicating a number of nonspecific, irritation induced changes, including epithelial desquamation, split formation, ciliacytophtoria and micropapillomatosis Metaplastic and hyperplastic alterations were more common in dust treated animals, while epithelial dysplasia was seen almost exclusively in carcinogen treated animals

Continued carcinogen treatment induced histologically detectable cellular atypia in the tracheobronchial epithelium with increas staining activity, as indicated by ninhydr gram and pyronin stains Cells of a vary density were present and exhibited abnormal wide intercellular spaces and desmosomes h unusually long filaments The cytoplasm co tained numerous lysosomes few mitochond and many filaments, singly or as den perinuclear bundles (Fig 25) Filamento granules were frequently seen in large cluste Enlarged nuclei were indented by deep cyt plasmic invaginations, giving them a poly bulated appearance Nucleoli were also enlarg and pleomorphic

Increased anerobic glycolysis as indicated strong lactate dehydrogenase activity, was se in certain areas The basement membrane w occasionally disturbed by cytoplasmic process of the epithelial cells which projected into t connective tissue layer (Fig 26) The relatio ship between metaplastic and dysplastic chang and subsequent malignant morphological alte ations similar to those seen in the bronchi a summarized in Table II



14 Increased number of hyperplastic cells in tobacco [illegible]
al epithelium (H—E x 450)
15 Extensive squamous metaplasia involving the epithelial [illegible]
16 Tracheal epithelium consisting of atypical cells with [illegible]
1600)
Metaplastic epithelium [illegible]

Table II *Morphology of Neoplastic Progression in Tracheobronchial Epithelium*

Class of Alteration	Metaplasia	Dysplasia	Squamous cell carcinoma
Histological changes	Regular squamous cells	Nucleocytoplasmic ratio increased	Large, irregular nucl
		Irregular stratification	Disorganized structu
	Ciliacytophtoria	Disturbed polarization	Cellular polymorphis
	Slit formation	Infrequent disintegration obasement membrane	Invasion into surrounding tissues
	Micropapillomatosis		
	Basal cell hyperplasia		
Histochemical alterations			
Staining activity			
Gram stain	Weak, irregular	Distinct	Distinct
Ninhydrin stain	Weak	Distinct	Very strong
Pyronin stain	Weak	Distinct	Distinct
Succinate dehydrogenase	Weak	Weak	Weak
Glucose 6-phosphate dehydrogenase	Irregular	Weak	Weak
Lactate dehydrogenase	Weak	Distinct, regular	Very strong irregular
Ultrastructure			
Intercellular relationship and attachments			
Intercellular space	Slightly widened	Widened	Widened
Desmosomes	Preserved	Decreased	Decreased, abnormal
Intercellular extensions	Few, regular	Increased	Numerous, irregular
Nucleus			
Shape	Regular	Polylobulated	Polylobulated
Nucleoli	Regular	Enlarged, pleomorphic	Enlarged, pleomorphic
Cytoplasm			
Tonofilaments	Increased	Increased, dispersed and perinuclear bundles	Increased, dispersed a perinuclear bundles
Lysosomes	Increased	Increased	Increased
Mitochondria	Preserved	Decreased, abnormal	Decreased, abnormal
Endoplasmic reticulum	Preserved	Decreased, rough increased, smooth	Increased smooth decreased, rough
Free ribosomes	Increased	Increased	Increased
Other organelles	Filamentous granules	Filamentous granules	Rare internalized cilia
Surface	Decrease in number of cilia	Atypical cilia	Few irregular cilia

Fig 21 Surface epithelial cells of carcinogen treated Syrian golden hamster showing abnormal trach cilia formation (Uranylacetate lead citrate x 23,200)

Fig 22 Multiple cilia and clublike thickened irregular structure in carcinogen treated tracheal epitheli (Uranylacetate lead citrate x 20,800)

Fig 23 Club shaped enlarget microvilli in carcinogen treated Syrian golden hamster tracheal epithelu (Uranylacetate lead citrate x 24 600)

Fig 24 Balloon shaped large and deranged microvilli in carcinogen treated Syrian golden hamster trache epithelium (Uranylacetate lead citrate x 25 000)

Fig 25. Abnormal mitochondria with intramitochondrial dense body in carcinogen treated Syrian gold tracheal epithelium (Uranylacetate lead citrate x 28 600)

Fig 26 Basal membrane disintegration and nuclear indentation in carcinogen treated Syrian gold hamster trachea epith [illegible] ranylacetate-lead citrate x 23,200)

4 Benign Tumors

Papillomas of tracheal epithelium were seen in 189 of 900 carcinogen treated animals, occasionally, one animal had several tumors These began as nodular epithelial proliferations, composed of regular squamous cells (Fig 27) Fully developed tumors consisted of proliferating squamous epithelium with a marked tendency toward keratinization and scanty connective tissue (Fig 28) Ultrastructural analysis revealed squamous cells with keratohyalin granules, large nuclei and numerous desmosomes (Fig 29) In some tumors a mucin containing columnar cell component was seen among the squamous cells (Fig 30)

Later occurring epithelial changes, such as irregular stratification and altered polarizati of the cells presented a spectrum ranging fro slightly atypical epithelium to changes reser bling carcinoma in situ Carcinoma in situ li changes in the epithelium consisted of irregul cells with hyperchromatic nuclei, freque mitosis and no evidence of stratification (Fi 31)

The papillomatous tumors occasionally showe features of polypoid tumors with scanty, muci secreting columnar cells Adenomatous lesio with mucus filled cystlike spaces were infreque (Fig 32) adenomas were seen in only animals Also infrequent were papillomato tumors consisting of fibrous stroma covere by regular squamous cells (Fig 33) and foun in 3 animals

Fig 27 Nodular lesion composed of proliferating squamous cells with intraepithelial keratin cysts i carcinogen treated Syrian golden hamster tracheobronchial epithelium (H—E x 130)

Fig 28 Tracheal papilloma composed of scanty stroma and proliferating epithelium (H—E x 62)

Fig 29 High powered view of squamous papilloma showing numerous tonofibrils keratohyalin granule and well preserved desmosomes (Uranylacetate lead citrate x 12 800)

Fig 30 Tracheal papilloma with columnar mucin containing cells in carcinogen treated hamster trache epithelium (H—E x 86)

Fig 31 Carcinoma in situ like lesions in tracheal epithelium consisting of tightly arranged polymorph cells (H—E x 115)

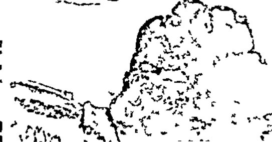
Fig 32 Polypoid lesion induced by repeated carcinogen applications composed of mucin countain glandular structures (H—E x 90)

Fig 33 Fibroepithelial tracheal tumor composed of proliferating stroma and regular squamous epithel (H—E x 90)









5 Malignant Tumors

The most common tumors were squamous cell tracheal carcinomas (127) These tracheal neoplasms were mostly well differentiated tumors, occasionally derived from benign papillomas The different types were similar to those of the main bronchi as described later They were frequently exophytic papillary tumors (Fig 34) which occasionally killed the animal by obstructing the airways Infiltration into the tissue surrounding the trachea was common Extension to the larynx and main bronchi was less frequently seen Metastases to the liver, kidneys and lymph nodes occurred infrequently, possibly because the animals died early in the experiment due to respiratory problems caused by the tumors

Morphological analysis of thin sections showed a consistency of polymorphic cells with large, indented nuclei surrounded by tonofilaments, well preserved intercellular bridges and widened intercellular spaces with multiple invaginations (Fig 35) The surface showe exfoliation of mature squamoid cells, like scale (Fig 36) Still, occasionally cells preserved the characteristics, i e, cilia (Fig 37) Stroma proliferation surrounding keratin cysts (Fig 38) was common

The number of nonkeratinizing tumors wa small The cells in these tumors were pleo morphic, with "light" and "dark" cells (Fig 39) Undifferentiated spindle cell tumors wer less common, and in these, ultrastructur analysis revealed an epithelial component (Fig 40) Sarcomas, composed of collagen producin atypical fibroblasts, were seen in 5 animals (Fig 41), while carcinosarcomas with a neoplasti squamous and fibrous component were un common A few adenocarcinomas originate in the tracheal epithelium, with well forme glands cribriform areas and solid areas o mucin containing structures (Fig 42) Smal "oat" cell type carcinomas were not observed i these studies The classification of tumors i similar to that shown in Table III

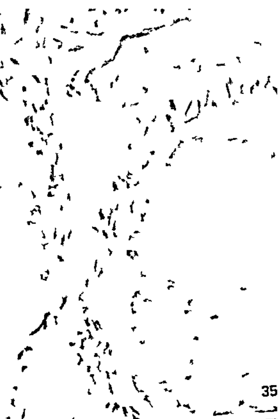
Fig 34 Papillary tumor showing numerous villous projections consisting of proliferating epithelial cells (Thiocarbohydrazide x 65)

Fig 35 Squamous cell carcinoma showing tonofilaments surrounding polymorphic nuclei with multiple projection and scanty desmosomes in widened intercellular spaces (Uranylacetate lead citrate x 12 000)

Fig 36 High powered view of squamous cell carcinoma with numerous keratin flakes (Thiocarbohydrazide x 180)

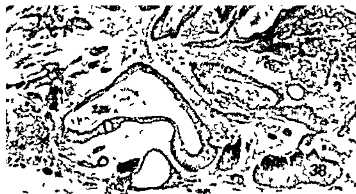
Fig 37 High powered view of the same tumor as in Fig 34 showing preserved microvilli but few ciliated cells (Thiocarbohydrazide x 180)




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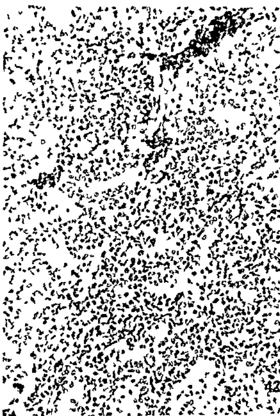














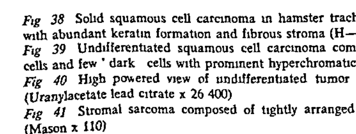


Fig 38 Solid squamous cell carcinoma in hamster trachea, induced by repeated carcinogen applications with abundant keratin formation and fibrous stroma (H—E x 28)

Fig 39 Undifferentiated squamous cell carcinoma composed of two cell types numerous large ' light cells and few ' dark cells with prominent hyperchromatic nuclei (Toluidine blue x 1000)

Fig 40 High powered view of undifferentiated tumor showing keratinization and intercellular bridges (Uranylacetate lead citrate x 26 400)

Fig 41 Stromal sarcoma composed of tightly arranged atypical fibroblasts invading surrounding tissues (Mason x 110)

Fig 42 Carcinogen induced Syrian golden hamster tracheal adenocarcinoma composed of glandular structures covered by columnar cells (H—E x 140)

B. Main Bronchi

1 Preneoplastic Conditions

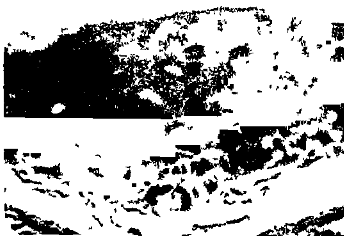
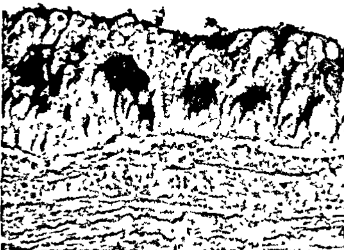
Untreated Syrian golden hamster bronchial epithelium is basically similar to that of the trachea with basal ciliated and mucous cells The main difference is the absence of cartilage, as well as of mucous glands

The neoplastic progression in the main bronchi of i t instilled hamsters was mostly similar to that seen in the trachea following two main pathways — squamous metaplastic and glandular proliferation and neoplastic trans formation

Initial changes in the tracheobronchial epi thelium were similar to those seen in the trachea, i e , lack of cilia, fragmentation and dissolution of epithelial cell and basal cell hyperplasia Increased cellularity, consisting of multiple layers of polygonal cells without differentiation into ciliated cells and mucous cells was also seen here (Fig 43) Epithelial proliferation gradually became irregular in character with disturbed stratification, nuclear polymorphism and hyper chromasia (Fig 44)

Another type of alteration consisted of mucous metaplasia with increased mucin content in the epithelium and a number of goblet cells which stained intensely with Alcian blue at pH 2 5 (Fig 45) Adenomatous hyper plasia was observed 5—6 weeks from the beginning of treatment, later becoming conspic uous with formation of pseudoglandular struc tures containing mucin secreting cells Ultra structural analysis revealed cyst like spaces with ciliary projections into the epithelium (Fig 46) Endophytic epithelial proliferation from the main bronchi into surrounding tissue, an obvious precursor of adenocarcinoma, occurred occasionally (Fig 47)






Figures 43—47

2 Benign Tumors

Benign tumors of the bronchial epithelium were basically similar to those in the trachea, though fewer number, 57 altogether. Most of these were composed of cuboidal cells at early stages and with scanty stroma (Fig 48) Higher magnification revealed pseudoglandular structures (Fig 49) Later-occurring lesions evidenced more obvious formations and an early disintegration of the basal membrane (Fig 50) In some papillomatous tumors mixed with the squamous cells, columnar mucus-containing cells were seen (Fig 51) Adenomas with glandular structures and mucin secretion were rare

3 Malignant Tumors

Neoplastic involvement of the main bronchi, occasionally concomitant with the trachea was observed in 117 animals and the average latent period was 42 weeks Tumor spread was sometimes extensive, grossly observed as nodules on the surface of the trachea, bronchi and lungs (Fig 52)

The largest group consisted of squamous squamous cell carcinomas which in these studies could be divided into 4 groups as follows 1) well differentiated tumors with keratin formation and horn cysts, 2) poorly differentiated tumors with eosinophilic polygonal cells and intercellular bridges but scanty keratin formation 3) anaplastic tumors composed of large cells with nuclei and coarse granular chromatin and small basophilic round cells, and 4) a spindle cell type, with few intercellular bridges, scanty cytoplasm and a sarcomatoid pattern

The well differentiated keratinizing tumors showed intercellular bridges, keratinization and genase activity, indicating increased anaerobic glycolysis (Fig 53) Nuclear chromatin, the staining of which varied from pale to dark, was often condensed into compact clumps and granular aggregates Desmosomes between adjoining cells were conspicuous and numerous The nucleocytoplasmic ratio was increased (Fig 54)

Nonkeratinizing, less differentiated squamous cell carcinomas (Fig 55) showed intercellular bridges intracellular keratohyalin granules and tonofilaments and deranged mitochondria Undifferentiated tumors studied by light microscopy showed two cell types — numerous large cells with nuclei containing coarse, irregularly distributed chromatin and rare "dark" cells with hyperchromatic nuclei (Fig 56) Metastases, mostly to local mediastinal lymph nodes, presented even more differentiated neoplasms (Fig 57) Mixed tumors were rare, although occasionally pseudoglandular squamous cell carcinomas were also encountered (Fig 58)

Fig 43 Slightly irregular epithelial hyperplasia in carcinogen treated Syrian hamster bronchial epithelium (H—E x 200)

Fig 44 Cellular atypia disturbed polarization and irregular stratification in squamous metaplastic bronchial epithelium in carcinogen-treated Syrian golden hamster (Toluidine blue x 800)

Fig 45 Hyperplastic bronchial epithelium showing intraepithelial accumulation of mucin (Alcian blue pH 2.5, PAS x 275)

Fig 46 Intraepithelial cystlike space with cilia in carcinogen treated hamster epithelium (Uranylacetate lead citrate x 24 600)

Fig 47 Glandular arrangements composed of cuboidal or polygonal cells around proliferating bronchial epithelium (H—E x 260)









Fig 48 Papillary projection into bronchial lumen composed of regularly built cuboidal cells (H—E x 180)
Fig 49 d m d cel s (H—F x 375)
Fg 50 40)
Fg 51 —E x 80)

Anaplastic carcinomas devoid of histologically detectable keratin formation were often composed of hyperchromatic cells with scanty cytoplasm (Fig. 59). Their latent period was shorter, 24 weeks on the average, as compared to 40 weeks for more differentiated tumors. Ultrastructural analysis revealed tonofilaments, which often formed fairly thick bundles, and keratohyalin granules and mitochondria, which were occasionally swollen with filamentous matrix (Fig. 60). Free ribosomal particles, lipid droplets, and occasional poorly developed Golgi apparati were present.

Adenocarcinomas of varying degrees of differentiation originating from the bronchi were also observed. The different types of these tumors as well as of other tumors observed are summarized in Table III.

Table III *Classification of Tracheal and Bronchial Tumors*

I. Trachea and main bronchi
  1 Papilloma
    a epidermoid
    b epidermoid with goblet cells
    c polyp like
  2 Adenoma
  3 Squamous cell carcinoma
    a keratinizing
    b nonkeratinizing
    c spindle cell
    d undifferentiated
  4 Adenocarcinoma
    a well differentiated
    b less differentiated
  5 Combined adeno/epidermoid
  6 Sarcoma
  7 Carcinosarcoma









Fig 52 Gross picture of carcinogen treated hamster respiratory tract showing extensive neoplastic involvement of trachea bronchi and lung

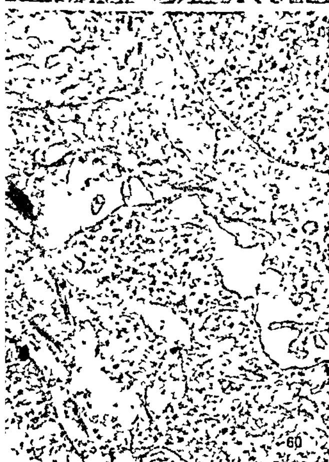
Fig 53 Lactate dehydrogenase activity of hamster squamous cell carcinomas showing peripheral active tumor cells surrounding central keratin core (Lactate dehydrogenase x 80)

Fig 54 High powered view of squamous cell carcinoma showing numerous tonofibrils abundant keratohyalin granules and nuclear indentations (Uranylacetate lead citrate x 28 000)

Fig 55 Non keratinized squamous cell carcinoma with hyperchromatic cells and scanty stroma (H—E x 180)

Fig 56 Hamster tumor formed by polymorphic large and small cells (Toluidine blue x 1000)





ig 57 Metastasis from keratinizing well differentiated squamous cell carcinoma into mediastinal lymph ode (H—E x 160)

ig 58 Squamous cell carcinoma with small polymorphic cells arranged in a glandular pattern in arcinogen treated hamster lung (H—E x 72)

ig 59 Sarcoma in hamster lung composed of hyperchromatic elongated cells in a whorl like pattern H—E x 180)

ig 60 High powered view of squamous cell carcinoma with papillary projections into dilated inter ellular space, deranged mitochondria and preserved tonofilaments and desmosomes (Uranylacetate-lead itrate x 6000)

had a monotonous uniform pattern of cuboidal tumor cells placed on delicate stromal septa, with continuous infolding and occasional large papillary projection into the lumen The nuclei were usually without mitoses and situated in the basal part of the eosinophilic cytoplasm Most frequently only one row of cells was seen on the delicate strands of connective tissue stroma but occasionally pseudostratification or two layers were present (Fig 67) Scattered, central ischemic necroses with focal calcification or cholesterol cleft formation were seen, sometimes both conditions were found simultaneously Mucus could not be demonstrated by periodic acid Schiff, Best's mucicarmine, or Alcian blue methods The surrounding lung often showed atelectasis with the appearance of a pseudo capsule, which did not serve as an effective barrier, since clusters of tumor cells could be found penetrating the atelectatic tissue

3 *Malignant Tumors*

Malignant bronchiolar tumors, 32 in all, mainly adenocarcinomas presented difficulty in definition On one hand, the malignant characteristics of bronchiolar adenoma like tumors were diffuse Cytological abnormalities varied, with no distinct differences, all the way to benign tumors and analysis of their relation to surrounding tissue was of disputable value, as benign tumors spread to surrounding alveoli On the other hand, providing the distinctive characteristics of malignant alveolar tumors was also difficult However, when sufficient TEM proof of participation of granular pneumocytes with lamellar bodies was given, a diagnosis of alveolar adenocarcinoma was possible

Most tumors presented a uniform picture of closely packed columns of cuboidal and columnar cells The cellular elements were supported by a sparse stroma of mature fibrous tissue containing small numbers of reticular and collagenic fibrils Few blood vessels were present The cells were arranged in acini, with papillary formations, and a stromal pattern, like that of epithelial growth (Fig 68) The cytoplasm of the tumor cells was generally smooth and slightly acidophilic, the nuclei were single, round or oval, and they varied from lightly stained vesicular to deeply basophilic types (Fig 69) Ultrastructural analysis indicated the existence of basal membrane like material between the tumor cells and cystlike spaces with ciliary projections into the lumen (Fig 70) Squamous cell carcinomas of definite bronchiolar origin were not observed

The types of tumors are summarized in Table V

Table V *Classification of Bronchiolar Tumors*

1 Adenoma
a papillary type
b acinar type
c solid type
d mixed type
2 Adenocarcinoma
a well differentiated
b less differentiated

Fig 66 Hamster papillary bronchiolar adenoma with projections covered by columnar epithelium (H—E x 130)

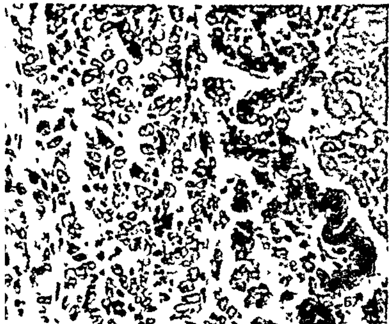
Fig 67 Bronchiolar adenoma composed of cuboidal cells (H—E x 180)

Fig 68 Bronchiolar adenocarcinoma with cytological abnormalities and preserved glandular features (H—E x 60)

Fig 69 High powered view of bronchiolar adenocarcinoma (H—E x 480)

Fig 70 Basal membrane like material and intraepithelial cyst formation in bronchiolar adenocarcinoma (Uranylacetate lead citrate x 26 400)






D. Alveoli

1 Metaplastic and Hyperplastic Lesions

In these studies, hamster alveolar cell proliferation was closely associated with treatment Dust treated animals occasionally displayed an increase in granular pneumocytes adjacent to dust accumulations (Fig 71)

A specific pattern consisting of extensive squamous metaplasia of the alveoli with keratin cysts (Fig 72), which rarely progressed to frankly invasive tumors, was occasionally observed in hamster alveoli This type of squamous differentiation of the alveolar cells consisted of large cells with regular nuclei and abundant eosinophilic cytoplasm Another type of squamous metaplasia associated with mucin formation was occasionally observed Solid areas of keratinized cells covered alveolar surfaces, sometimes obliterating the lumen (Fig 73) They occurred close to bronchioli without direct connection

Specific non neoplastic lesions were the localized mucous lesions These circumscribed lesions contained scanty cells, adjacent to a peripheral bronchiole (Fig 74) Occasionally they were diffuse lesions with little cell proliferation and abundant mucin formation (Fig 75) A lesion peculiar to this experiment, originating in small islets of cuboidal cells in the alveoli (Fig 76), these lesions progressed to s c adeno squamous lesions (Fig 77), composed of squamous cells arranged in a glandular fashion Cords or islets of tightly arranged small cells presented rounded basophilic nuclei faintly staining cytoplasm and distinct cell borders (Fig 78) The superficial cell layers were occasionally flattened, however, horn pearl, keratinization, or unequivocal intercellular bridges rarely occurred Some lesions showed papillary or acinar structures consisting of cuboidal basophilic cells, rarely showing secretory activity The lesions were always clearly delineated with no signs of infiltration into lung tissue or formation of metastases

The relationship of alveolar lesions to inducing agent is summarized in Table VI showing the increased incidence of metaplastic lesions in carcinogen treated animals though specific changes indicating subsequent tumor formation or exclusively found in carcinogen treated animals were not observed

Table VI *Relation of Alveolar Lesions to Inducing Agent*

Type of lesion	Treatment		
	None	Dust	Carcinogen
Alveolar cell (Type II proliferation	+	+++	++
Squamous metaplasia	+–	+–	+
Mucous metaplasia	+–	+–	+
Squamous/mucous metaplasia	+–	+	+
Keratinization	+–	+–	+
Mucous lesions	+	+	+
Adenosquamous lesion	–	–	+
Squamous metaplastic lesion	+–	+–	+–
Nodular hyperplastic lesion	–	+–	+

Occurrence
– = non existent
+– = very rare
+ = infrequent
++ = regular
+++ = very common

Fig 71 Nodular lesion composed of granular pneumocytes surrounding Fe_2O_3 dust accumulation (H—E x 75)

Fig 72 Alveolar squamous metaplasia of Syrian golden hamster showing large cells with monomorphic nuclei (H—E x 250)

Fig 73 Intense squamous metaplasia with keratin formation adjacent to bronchus of Syrian golden hamster (H—E x 80)

Fig 74 Mucous lesion in Syrian golden hamster Note clearly demarcation with abundant mucin and scanty cells (H—E x 45)



Figures 71—74

2 *Benign Alveolar Tumors*

This tumor type, seen in 7 % of the animals, began as an accumulation of small cuboidal cells frequently close to the pulmonary surface (Fig 79) Those most commonly encountered consisted of solid, closely packed, curving, and sometimes sinous columns of cuboidal cells whose intercellular boundaries were often prominent and gave the impression that the cells were forceably pressed together (Fig 80) A second type revealed recognizable alveoli in various stages of collapse and were completely invested by single or multiple layers of cuboidal cells similar in every respect to those of the more solid tumor type Thus the common pulmonary tumor was seen to arise through a preliminary proliferation of alveolar cells that occurred throughout the lung tissue, but was prominent in the peripheral alveoli remote from any contact with bronchioles or bronchi Ultrastructural analysis showed the presence of multicentric osmiophilic lamellar bodies (Fig 81)

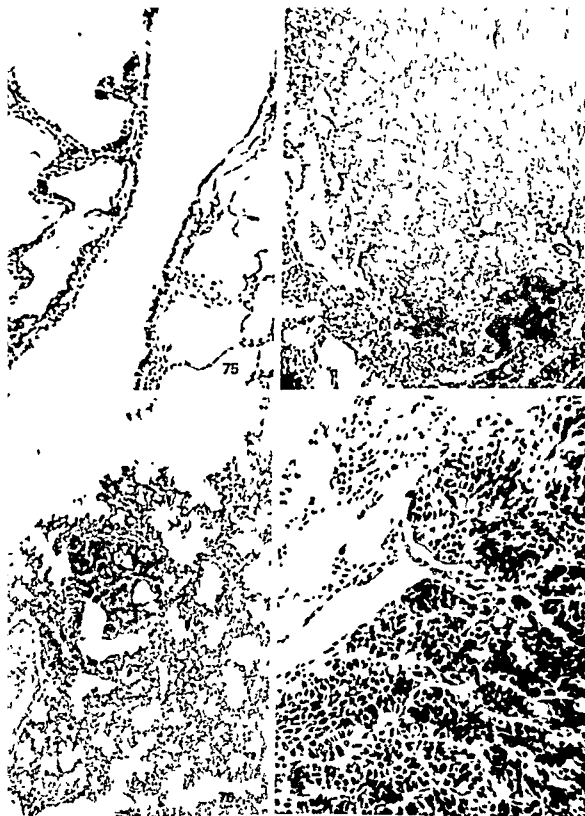
3 *Malignant Alveolar Tumors*

Neoplastic involvement of the peripheral lung was seen in 224 animals Most frequent were adenocarcinomas which could be divided into three groups, depending upon their degree of differentiation *Well differentiated* tumors presented glandular structures of varying sizes, covered by alcianophilic columnar epithelium (Fig 82) The epithelial cells were arranged in glandular structures, with a basal, enlarged nucleus and pale cytoplasm (Fig 83) Cells varied from cuboidal to columnar in shape (Fig 84) A rare type consisted of mucinous tumors in which secretion products were easily recognizable (Fig 85) Epithelial proliferation with mucin accumulation occurred in well differentiated tumors Papillary tumors consisted of proliferating cylindrical epithelium, with a solid, fibrous stroma The cells stained intensively for mucin Alcian blue pH 2 5, as well as azure A pH 5 and $MgCl_2$ 0 1 % Decreasing the pH of azure A or increasing the concentration of $MgCl_2$ abolished the staining, except for the surface cell borders Ultrastructural analysis showed nuclear polymorphism with aggregates of pale-to-dark staining chromatin dispersed inside the large nucleus Microvilli, acinar formations, desmosomes, and terminal bars were observed Parallel profiles of granular endoplasmic reticulum with occasional dilatation to form cisternae were abundant and occasionally surrounded mitochondria with numerous lamellar bodies (Fig 86) Fairly well developed Golgi complexes were numerous, particularly in more differentiated forms, and had secretory vacuoles full of loose fibrilar and globular material, resembling that in mucin secreting glandular epithelium

Squamous metaplasia of adenocarcinomas was rare and only 4 true mixed tumors, with neoplastic squamous and glandular structures, were encountered Extension from proximally located squamous cell carcinomas was common although peripheral squamous cell carcinomas not associated with bronchi or bronchioli were less frequent They were mostly composed of squamous cells with individual dyskaryosis and

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Fig 75 Metaplastic alterations with squamous cells lining alveoli walls and mucin in lumen (H—E x 120)
Fig 76 Beginning adenosquamous lesion adjacent to bronchiolus in carcinogen treated hamster lung (H—E x 52)
Fig 77 Adenosquamous lesion of Syrian golden hamster composed of squamous cells in a *glandular* fashion (H—E x 72)
Fig 78 High powered view of adenosquamous lesion showing glandular structures covered by cuboidal or flattened cells (H—E x 200)



ıres 75—78

Table VII *Classification of Alveolar Tumors*

I Benign tumors
  1 Adenoma
    a cuboidal cell type
    b solid type
II Malignant tumors
  1 Adenocarcinoma
    a well differentiated
    b less differentiated cuboidal cell type
    c mucin producing
  2 Squamous cell carcinoma
    a keratinizing
    b undifferentiated
  3 Combined adeno/epidermoid carcinoma

cytological abnormalities devoid of keratin cysts (Fig 87) Undifferentiated sarcomalike tumors similar to those previously described were seen in only two animals The types of tumors are summarized in Table VII

E Classification of tumors

As previously presented the tumors can be classified according to standard histopathol ogical criteria and compared to those in man (Table VIII) The two main groups are squamous cell carcinomas and adenocarcinomas accounting for 47 % and 20 %, respectively of all tumors The main exception is the absence of small cell anaplastic carcinomas not seen in these studies, nor were carcinoid tumors or bronchial gland tumors observed Frequent findings were papillary tumors of the surface epithelium (21 %) Carcinosarcomas and sar comas constituted less than 3 % of all tumors Adenomas of bronchiolar or alveolar origin respectively, were observed in 9 % of the hamsters but rarely are seen in man Large cell tumors as seen in man were in this study analyzed by histological histochemical and ultrastructural methods and classified according to the main component whether squamous or adenomatous in nature

Fig 79 Accumulation of numerous regular alveolar cells adjacent to pleural surface (H—E x 150)
Fig 80 Alveolar adenoma composed of small regular cuboidal cells in glandular arrangements (H—E x 45)
Fig 81 Osmiophilic lamellar bodies in Type II alveolar cells in alveolar adenoma of Syrian golden hamster (Uranylacetate lead citrate x 26 000)
Fig 82 Profuse mucin secretion in well differentiated adenocarcinoma showing abundance of acid and neutral mucosubstances (Alcian blue pH 2 5 PAS x 430)

Table VIII *Classification of Experimentally Induced Respiratory Tumors as Compared to the WHO Classification*

Experimental	WHO
Epidermoid ca keratinizing nonkeratinizing spindle cell undifferentiated	I Epidermoid carcinoma
[Not seen]	II Small cell anaplastic carcinoma
Adenocarcinoma Bronchial Bronchiolar Alveolar	III Adenocarcinoma 1 Bronchogenic a acinar b papillary 2 Bronchioloalveolar
[Classified by their differentiated component]	IV Large cell
Combined adeno/epidermoid	V Combined adeno/epidermoid
[Not seen]	VI Carcinoid
[Not seen]	VII Bronchial gland tumor
Papillary tumors of the surface epithelium Epidermoid Epidermoid with goblet cell Polyp like	VIII Papillary tumor of the surface epithelium Epidermoid Epidermoid with goblet cell Others
Carcinosarcoma	IX Mixed tumors and carcinosarcomas 1 Mixed tumors 2 Embryonal blastomas
Sarcoma	X Sarcoma
Adenoma bronchial bronchiolar alveolar	XI Unclassified

Fig 83 Alveolar adenocarcinoma composed of large columnar cells arranged in a glandular pattern (H—E x 115)

Fig 84 Columnar cells with large nuclei and scanty mucin formation (H—E x 200)

Fig 85 Adenocarcinoma formed of abundant mucin producing cells (H—E x 150)

Fig 86 Numerous lamellar bodies in carcinogen induced alveolar adenocarcinoma (Uranylacetate-lead citrate x 26 000)

Fig 87 Squamous cell carcinoma in hamster lung (H—E x 400)

V DISCUSSION

A. Preneoplastic Lesions and Their Significance

1 *Trachebronchial epithelial alterations*

Several studies stressed the significance of epithelial metaplasia for the development of bronchial cancer (Niskanen, 1949, Auerbach *et al*, 1957a, 1957b, Valentine, 1957, Sanderud, 1958, Cross *et al*, 1961) Fully developed, keratinized squamous epithelium was rare in non cancerous bronchial epithelium in autopsies of humans Hamilton *et al* (1957), Cunningham & Winstanly (1959), and Carrol (1961) have stressed that keratinization is unusual in squamous metaplasia Conversely, squamous *metaplasia is a common finding in non neoplastic diseases of the lung* (Niskanen, 1949)

Exposure of respiratory epithelium to chemical carcinogens has been shown to cause atypical hyperplasia and squamous metaplasia (Frasca *et al* 1968, Auerbach *et al* 1970, Harris *et al*, 1971) Harris *et al* (1971), using a $B(a)P/Fe_2O_3$ mixture in hamsters, reported that squamous metaplasia and hyperplasia occurred only when both agents were used together Squamous metaplasia has been considered a stage in the tumorigenesis of squamous cell carcinoma (Auerbach *et al* 1961, Saffiotti *et al*, 1968) Warren & Gates (1968) did not, however, observe squamous metaplasia in association with their radiation induced epidermoid carcinomas of mouse lung

Other features characteristic of carcinogen treated hamster tracheobronchial epithelium include slit formation and expulsion, which exist in inflammatory states in the lungs of man (Nasiell, 1963), producing so called "ciliocytophtoria" or cell destruction (Papanicolau, 1956, 1958, Pierce & Hirsch, 1958, Pierce & Knox, 1960) In humans the frequency of lung cancer was doubled in patients with ciliacytophtoria in bronchial smears compared to those without it (Nasiell, 1963, 1968) It was also suggested that slit formation and expulsion acted as a preliminary step prior to squamous metaplasia

Evaluation is made more difficult by the large number of descriptive terms applied to the various metaplastic and hyperplastic lesions e g , basal cell hyperplasia, squamous atypism, *adenomatous hyperplasia, reserve cell hyperplasia*, transitional cell metaplasia, squamous *metaplasia*, stratification, metaplastic polymorphous epithelium, *pretransitional* epithelium and micropapillomatosis Weller (1954) has divided the hyperplasia into proliferative and dormant types, and Wittekind & Struder (1953) have reported a type I and a type II hyperplasia

Ciliary abnormalities in association with bronchiogenic carcinoma have been described in man (Ailsby & Ghadially, 1973) As these abnormalities are likely to impair motility and direction of ciliary beat (Fawcett, 1961), they may have significance for the location and persistence of the carcinogen Compound cilia compound bodies, and numerous basal bodies were considered by Harris *et al* (1974) to be nonspecific ultrastructural lesions occurring during carcinogenesis in the respiratory tract

Structural abnormalities in surface ciliary structures have been observed in nitromethyl urea (NMU) treated animals and in animals receiving B(a)P/Fe_2O_3 (Harris *et al* 1974) Loss of cilia abnormal cytoplasmic projections between cilia and evidence of ciliogenesis were observed Filamentous granules have also been seen following treatment either with cigarette smoke or B(a)P Fe_2O_3 Filamentous granules however were *not* seen in squamous metaplastic cells caused by vitamin A deficiency (Harris *et al* 1972) Loss of ciliated cells with broad focal areas of epithelial hyperplasia were observed by SEM in B(a)P/Fe_2O_3 treated hamsters

The metaplastic changes described here may promote carcinogenesis by inhibiting adequate tracheobronchial clearing and removal of the causative agents as well as facilitating absorption and penetration of the carcinogen and permitting chemical interaction between the carcinogen and the lining cells Continuing the carcinogen treatment for prolonged periods of time i e with B(a)P and Fe_2O_3 produced atypical epithelial changes histologically and histochemically related to dysplasia of the cervical epithelium (Scarpelli & von Haam 1960 Stenbäck 1970) A formal relationship is suggested between these tracheobronchial epithelial lesions and their progression to severe dysplasia and carcinoma in situ This phenomenon has been observed in the bronchi of humans and in some animal experiments (Auerbach *et al* 1957a 1957b Black & Ackerman 1952)

Changes in nuclear structure remain one of the most reliable criteria for morphological diagnosis of neoplasia Enlarged irregular nuclei were an early finding following NMU treatment (Harris *et al* 1974) Pleomorphic nucleoli were often enlarged and macrosegregated in carcinogen treated animals (Svoboda & Higginson 1968)

Defects in the basement lamina in areas of squamous metaplasia have been shown in NMU treated animals though not as numerous as those following instillation of B(a)P/Fe_2O_3 (Harris *et al* 1971, 1973) It was postulated that these defects do not represent only the acute [illegible] ould be fur [illegible] th [illegible] en [illegible] [illegible] ration of the carcinogen [illegible] reflection of dysfunction of [illegible] related to the morphogenesis of sq[illegible] cell carcinoma Defects in the basement lamina have also been described in premalignant and malignant lesions of the mammary gland (Ozzello & Sanpitak 1970 Tarin 1969) hamster cheek pouch (Woods & Smith 1969) and larynx (Sugar 1969) A disruption of the basement lamina is not specific for carcinogenesis since similar lesions have been observed in psoriasis (Cox 1969)

Repeated carcinogen application in experimental skin tumorigenesis has also produced similar lesions (Sugar 1969 Tarin 1967) such as cellular pleomorphism disturbed cellular arrangements loss of contacts of basal cells and hyperplasia These are considered sequential changes preceding frank carcinoma and have also been observed in the cervix (Scarpelli & von Haam 1960) while in the bronchi a finding of carcinoma in situ has been considered strongly indicative that an invasive carcinoma will arise (Stout 1960) The spatial relationship and their simultaneous occurrence in the same specimen with malignancy speaks in favor of a causal relationship

The histochemical analysis in this study included three different aspects 1 attempt to find histochemical changes specific to malignant transformation 2 histochemical changes with prognostic significance indicating subsequent tumor transformation prior to morphologically detectable neoplastic changes and 3 aid in the classification of premalignant lesions and tumors In this study the histochemically detectable changes in keratinization cell differentiation and maturation accompanied but did not precede histologically observed lesions Histochemically specifically enzyme histochemical changes in activity and distribution of oxidative enzymes were also observed as shown in skin (Stenbäck 1970) Histochemical characterization of mucins were also helpful in differentiation of tumors as discussed later

Peripheral bronchial and bronchiolar pre

man (Obiditsch Mayer & Breitfellner, 1958, Razzuk *et al*, 1970)

4 Location of Tumors

A certain correlation was also found between inducing agent and type as well as location of tumor (Stenbäck, 1974) This also relates to dose of carcinogen as well as survival, showing that if the animals do not succumb from other causes, malignant respiratory tumors are found in 100 % of them

As shown in these studies, tracheal tumors are readily induced by i t instillation of carcinogens However, in man, the occurrence of tracheal carcinomas has not paralleled the increasing rate of lung and laryngeal carcinoma (Houston 1967, Ranke *et al*, 1962), although a positive correlation between cigarette smoking and development of epidermoid carcinoma of the trachea has been reported (Acquarelli *et al*, 1967, Hajdu *et al*, 1970) The rarity of human tracheal carcinomas has been explained by the simple structure, immobility and passive function of the trachea (Chevallier, 1930) Other explanations are that a vigorous cough reflex, more effective cleansing ciliary action, and less trapping of mucus spare the trachea from prolonged contact with mucus and the carcinogenic agent (Hilding, 1956, Ranke *et al*, 1962)

C Intratracheal Instillations into Syrian Golden Hamsters as a Model System

The usefulness of a model system, such as that described, depends on several factors, efficiency, reliability, simplicity and similarity to the actual conditions it is supposed to reflect

Intratracheal instillation of polycyclic hydrocarbons into hamster respiratory tract in this study has proved to possess several benefits, compared to other methods The tumor response is fairly rapid and the incidence, high The process affects different cellular constituents in all parts of the respiratory tract

The Syrian golden hamster also proved useful for this type of study The respiratory tract is, in many aspects, similar to that in man the main difference being a sparsity of bronchial glands in the lower parts of the tract and an absence of supporting structures, such as cartilage, in the bronchi The spontaneous tumor incidence is low, consisting only of peripheral proliferative lesions with questionable neoplastic significance Malignant epithelial tumors are virtually unknown The hamster is also a hardy animal and tolerates large amounts of carcinogens and severe treatment, while the side effects are small and the incidence of respiratory infections low The similarity of the neoplastic process to that seen in man is one of the main subjects for the present study This relates both to the mode of the progression of the neoplastic process and types of tumors formed, as well as preneoplastic and premalignant conditions

VI SUMMARY

Respiratory tumors and their precursors induced by polycyclic hydrocarbons in the lungs of Syrian golden hamsters were studied in terms of their formation and specificity and compared to non-neoplastic lesions and lung tumors in man. The purpose was to further delineate and define the validity of the i.t. instillation model and to gain understanding into the causation and behavior of this type of neoplasm in terms of morphology, treatment and prognosis as well as classification of these tumors.

In hamsters, the early metaplastic changes in the tracheobronchial epithelium caused by carcinogen instillation were similar to those in humans. These included squamous and transitional cell metaplasia, lack of cilia, formation of club shaped or multibranched cilia, dysplastic alterations, cytological abnormalities, widened intercellular spaces with villous interdigitations and sparse desmosomes.

Unique changes indicative of subsequent tumor formation or exclusively associated with carcinogen treatment in early stages were scarce but a significantly increased incidence of metaplastic and hyperplastic changes were observed prior to the manifestation of visible tumors.

These alterations were followed by a large number of neoplastic lesions, mainly tracheal papillomas. The most frequent malignant tumors in hamsters were squamous cell carcinomas of a varying differentiation: keratinizing tumors, less differentiated tumors with intercellular bridges, polygonal cell tumors without keratin cysts but with tonofilaments and keratohyalin granules, as well as a spindle-cell type with few intercellular bridges and prominent connective tissue formation. Careful analysis, including ultrastructural studies frequently revealed anaplastic and large cell tumors to be squamous in character. Squamous cell carcinomas of bronchiolar origin were difficult to distinguish, alveolar squamous cell proliferation and tumor formation were less common.

Tracheobronchial hyperplasia, mucus metaplasia, intraepithelial cyst formation and pseudoglandular epithelial structures preceded epithelial adenocarcinoma formation. These tumors could be divided into two groups depending upon the degree of differentiation. Mucin production was mostly scanty, profusely mucin producing tumors were uncommon. Ultrastructural and histochemical analysis was necessary to define anaplastic and large cell tumors belonging to this group, however the cell type of origin was difficult to determine. Bronchiolar neoplasms developed three different cell patterns, i.e., papillary, acinar and solid tumors. Alveolar epithelial proliferation produced tumors containing type II alveolar cells with abundant cytoplasmic lamellar structures.

A total of 852 tumors were observed in this study, 34 % of these were benign and 66 % malignant. The average latency period was 42 weeks, undifferentiated tumors having a shorter latent period, this was not dependent upon tumor location. Of these tumors 37 % were located in the trachea, 20 % in the main bronchi, 9 % in the bronchiolar area and 34 % in the peripheral

lung though extensive involvement of large segments of the respiratory tract was not in frequent

The classification used in this study, based on morphological and histogenetical aspects, differed mainly in emphasizing the degree of differentiation as well as the cell type of origin and localisation of tumors The WHO classi fication with some modifications was also applicable to this experimental system

The results showed that i t instillations of car cinogens induced a large number of tumors of different types which are also found in man Morphological analysis revealed an association between the rate of progression, tumor growth metastasis, morphological appearance and biological behavior and preceding non neoplas tic alterations or cell type of origin as well as location Certain histochemical and ultra structural tracheobronchial, bronchiolar and alveolar changes were also discerned

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SUPPLEMENT 348

New Methods for the Recording of the Eustachian Tube Function

BY

MARCUS DIAMANT, M.D.

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SUPPLEMENT 348

New Methods for the Recording of the Eustachian Tube Function

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Motto

"The difficulty is less in discovering than in having discoveries understood and adopted" (Pasteur)

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Introduction

The function of the human Eustachian tube presents quite a number of mutually entangled and still unsolved medical problems Its purpose of providing for the indispensable ventilation of the closed middle ear has never been studied systematically, i e step by step from the very basic physiology Indeed, practically all fundamental problems still remain "known" only in stages of scientifically unsupported and contradictory hypotheses They are often evasively claimed to be "generally accepted". However, medical problems cannot be solved by the mere acceptance of the opinions of some majority, but only by adequate and scientific research studies

Five new methods for recording the tubal ventilatory function are to be presented They have been designed in order to overcome the shortcomings of the currently used methods

PART I

Problems of Middle Ear Ventilation

Primary factors of the middle ear ventilation

Fundamentally, the middle ear air spaces resemble non-ventilated subcutaneous air pockets of the human body (1) The sum of the partial gas pressure of all constituents of the enclosed atmospheric air (with their mutually differing percentages of O_2, N_2 and CO_2) is greater than that of the capillary blood in the locally lining mucosa Thus, a transfer of gas (and above all of oxygen) proceeds from the air to the blood (2, 3, 4) (Under certain circumstances some fluid passes in the opposite direction)

The gas resorption in the middle ear is unceasingly maintained, irrespective of an intact or perforated tympanic membrane and also irrespective of a permanently or only intermitt- y open Eustachian tube However, gas -orption causes a decrease of the middle ear -essure only when the middle ear air space *is closed, i e. while an intact (or healed)* tympanic membrane co-exists with a closed tubal lumen Such a decrease of middle ear pressure causes a simultaneous decrease of middle ear volume only when the intact tympanic membrane is movable and thus can deviate from its "neutral" position or when the middle ear mucosa reactively increases its thickness (31)

The continuous gas resorption in the middle ear necessitates an access of sufficient quantities of substituting air In ears with a tympanic membrane perforation or with symptoms of a clinical tuba aperta (or without subjective symptoms of a physiologically permanently open tubal lumen), the indispensable re-aeration can proceed continuously In clinically healthy ears with an intact tympanic membrane and an intermittently closed and open tubal lumen, the re-aeration can only occur intermittently In the relevant literature (5, 6, 7, 9) the closed Eustachian tube is representing a state of "passive resting" and the open tubal lumen a state of "active working" The proper activity for the opening of the passively closed tubal lumen is attributed to ordinary (or to additional voluntary) swallowings (7, 34)

Consequently, the ventilatory tubal mechanism is exclusively responsible for the indispensable re-aeration of the closed middle ear Under local physiologic conditions this *responsibility* also implies a continuous maintenance of middle ear pressure and volume within certain border limits In turn, this prevents from subjective disturbances from the middle ear region (including the hearing decrease) These border limits are still non-assessed in quantitative recordings Thus, no physiologic or non physiologic measurement actually recorded can be properly evaluated as for its clinical significance, neither prognostically nor therapeutically

PART II

The Current Methods of Recording the Eustachian Tube Functional Patency

Different methods of recording the Eustachian tube function

The *qualitative* Valsalva test (22) has been used for centuries to differentiate between an open and a closed tubal lumen. Only recently has the *quantitatively* recorded tubal air flow capacity become an increasing *clinical* interest. In *healthy* ears this information is needed to judge the fitness of a prospective pilot or a marine diver. In *diseased* ears it is claimed to be useful when selecting cases for myringoplasty by predicting the chances of a post operative transplant healing (28).

The impedance and tympanometry methods (66–70, 39, 47, 48, 57, 75, 79, 81) as well as the volumetric methods (5–9, 27–34, 41–46, 56, 58–60, 49, 51) are basically recording the effects of controlled pressure changes in the closed (or occluded) middle ear air spaces. A pre-requisite for all these methods is some remaining tubal patency as well as a movable tympanic membrane (its deviations automatically changing the volume of the closed middle ear air spaces). However, the acoustic impedance and tympanometric methods are explicitly studying the effects of local *pressure* changes on the *sound transmission system*. They do not aim to quantity measures of tubal air passage capacity or of measures of middle ear volume. In fact, the deviations of the intact tympanic membrane are partly caused *passively* by pressure changes and partly by the *activity* of the tensor tympani muscle. It has recently been shown that "experimental section of the tensor tympani muscle (the muscle actively influencing deviation of the tympanic membrane and thus volume of the middle ear) does not influence the acoustically recorded tympanogram" (71).

Current volumetric methods of recording the tubal patency

The current volumetric recordings of the tubal air flow capacity were first suggested by the Ingelstedt group. The methodology and equipment have been supplemented step by step, and some of the advanced instruments for the numerous and elaborate studies were contrived by the authors themselves for this purpose (31, 32, 42).

The instrumental equipment consists of

a) pressure chamber with "snorkel" arrangements (Fig. 1),
b) catheter with an inflatable rubber cuff device (Fig. 2) for the airtight lodging in the bony part of the external ear canal (Fig. 3),
c) manometer (closed system),
d) air flow meter device (open system) (Fig. 4*a* and 4*b*), and
e) quite a number of additional electronic and other highly advanced measuring devices as well as time and graph recorders (Fig. 5).

Furthermore, in two studies aiming at *direct* recordings

f) a bone puncturing mandrin needle (Fig. 6).

Recording arrangements

Basically, the current volumetric methods follow a uniform pattern in quantitatively recording the changes of volume and pressure in the clinically healthy as well as in diseased ears. *Prior* to the start of the proper measurings some preparatory arrangements are

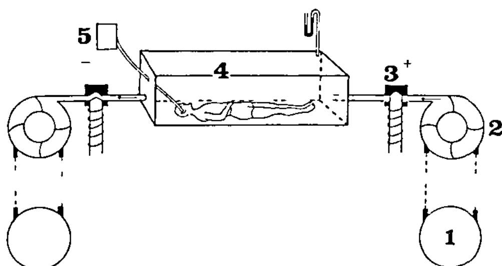


Fig 1 Ear snorkel–pressure chamber device 1, Autotransformer, 2 fan 3 electromagnetic valve 4 pressure chamber, 5 flow indication device (rate meter) (From Ingelstedt & Örtegren (42) *Acta Otolaryngol* Suppl 182 1963)

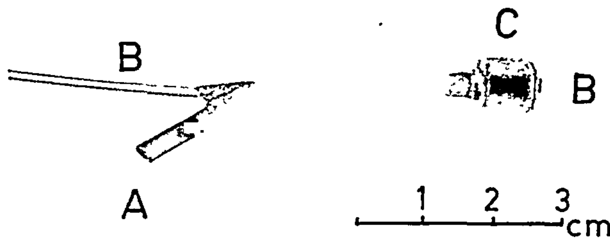
1 the airtight lodging of the catheter with its inflated rubber cuff device in the bony part of the external ear canal,

2 the election of some negative or positive pressure level to be attained in the pressure chamber, and

3 the imposing of the pre-elected pressure level in the closed middle ear or in the occluded proximal space of the external ear canal (thus in the clinically healthy ear accordingly deviating the tympanic membrane from its "neutral" position)

Voluntary consecutive deglutitions are now started and continued Each tuba-opening effect is repeatedly allowing a small volume of air to pass through the tubal lumen

In clinically healthy ears the thus *deviated* position of the tympanic membrane is constituting the starting point, and its re-established "neutral" position the comple-



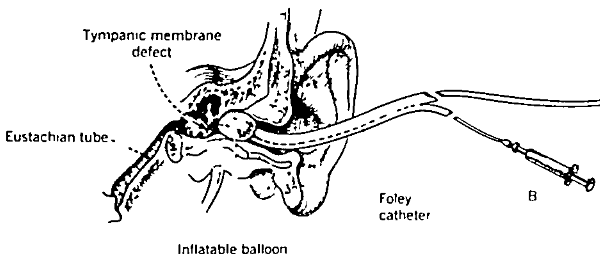


Fig 3 B Syringe for the glycerine inflat on into the t ght ening balloon (From Siedentop Ham lt n & Osenar (76) *Arch Ot laryng 195* 1972)

tion of the measuring recording For obvious reasons each position change of the tympanic membrane causes simultaneous changes of the gas volume in the closed middle ear as well as in the occluded part of the external ear canal

The current volumetric methods of record ing the air passage capacity of the tubal lumen are thus measuring volume changes at

1 the *re equilibration* of a previously and preparatorily introduced pressure level
2 the *re establishing* of the neutral position of the previously and intentionally deviated tympanic membrane
3 the *restoring* of the previously and automat ically caused volume change of the closed middle ear air space and
4 the co-ordinated *restoring* of pressure and volume changes as mediated into the oc cluded space of the external ear canal by the deviation of the tympanic membrane

From this latter space all the volumetric measurings are recorded to be representative

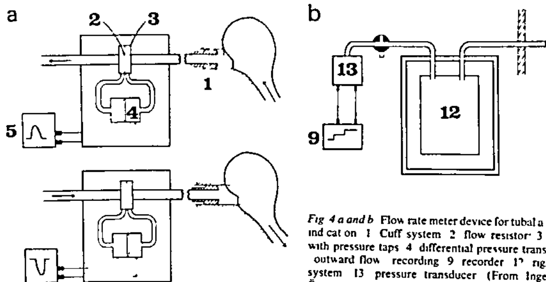
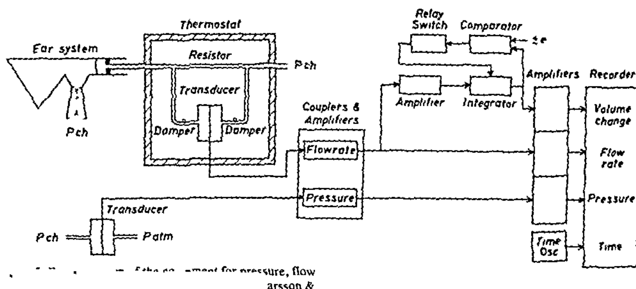


Fig 4 a and b Flow rate meter device for tubal a r passage ind cat on 1 Cuff system 2 flow resistor 3 cylinder with pressure taps 4 differential pressure transducer 5 outward flow recording 9 recorder 1? rigid closed system 13 pressure transducer (From Ingelstedt & Örtegren (28) *Acta Otolaryngol* Suppl 18?)



. . . . f the equipment for pressure, flow
arsson &

of the simultaneous air passage through the tubal lumen

For several reasons the number of deglutitions needed during the quantitative study may vary considerably When used as a parameter of Eustachian tube function an increased number of deglutitions is considered tantamount[1] to an increased abnormality of the ventilation of the closed middle ear (8)

Due to the non physiologic nature of the method-related *intermittent* release of the pre-deviating movements of the tympanic membrane at the deglutitions, i e the repeated rapid and shortly lasting effects of the tubal lumen opening, each change of position of the tympanic membrane presents itself as an air puff or an air sucking (depending respectively on the preparatory imposing of a negative or a positive pressure level into the closed middle ear) Each resultant air flow always has a one-way direction (proximal–distal or distal–proximal) Thus each sucking inwards ("aspiration") through the tubal lumen causes an air puff directed outwards in the external ear canal, and vice versa

[1] Voluntary deglutitions have been shown to open the tubal lumen only irregularly (. . . . .) even during five deglutitions in a row) (. . .) rthermore the tubal opening effect has been shown . . . . short lapses of time (around 0 12–0 61

Management of the current volumetric method

The catheter with its rubber cuff device (Fig 2) is lodged in the bony part of the external ear canal (Fig 3) and then inflated for the air tight occlusion of its proximal site The distal end of the catheter is then directly connected to the air flow meter device (Fig 4) This sensitive recorder allows measures of one microliter (0 001 ml), with a standard error averaging ±5 per cent

All recordings are made numerically and simultaneously in time and graphs Each deglutition is also demarcated until reaching the completion of the study The sum of the air volumes measured indirectly and step by step thus corresponds to the volume change of the middle ear originally caused by the preparatory imposing of the pre-elected pressure from the pressure chamber, as shown in Fig 7

The basic principles of the current volumetric methods for the recording of the tubal air passage capacity are based on computations according to the general gas law by Boyle (7, 30, 43, 85) They are presented as follows (31)

'Several variables must be determined for a reliable measuring of the air through the tube (ΔV_t) the volume of the middle ear space (V_m) the pressure changes in the closed mid

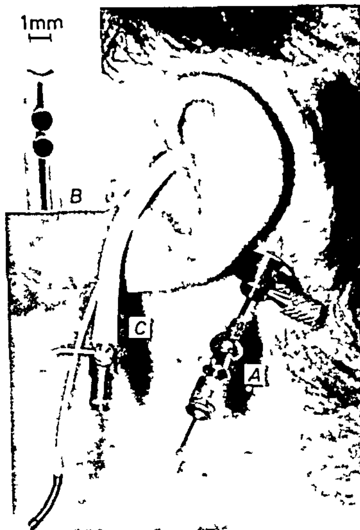


Fig 6 The puncturing mandrin needle at the recording of the ventilatory effects from the cuff device lodged in the external ear canal (according to the Ingelstedt group recording method) *A* Puncturing needle inserted *B* detail of needle *C* cuff system (From Flisberg Ingelstedt and Örtegren (??) *Acta Otolaryngol* Suppl 18? 1963)

dle ear cavity (ΔP_m) and finally the degree of the ear drum mobility measured as a volume displacement (ΔV_{tm}) which is a function of ΔP_m

(Note that the Δ placed in front of a symbol indicates a change of this variable)

When quantitatively recording in ears with a tympanic membrane *perforation* the air flow volume (at each voluntary deglutition) is measured *directly* from the preparatorily occluded space of the external ear canal By connecting the catheter to the air flow meter device the measurements are made in an open measuring system (42)

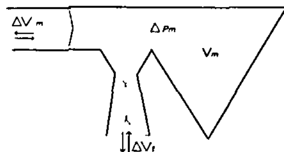


Fig 7 Schematic middle ear model Variables to be determined in the middle ear system V_m Volume of ear space ΔP_m pressure changes within the ear space ΔV_{tm} volume displacement of the tympanic membrane ΔV_t rate of tubal ventilation (From Ingelstedt & Jonson (31) *Acta Otolaryngol* Suppl 224 1967)

Discussion

According to the principles of the current volumetric methods

1 the volume of air passing through the tubal lumen quantitatively reflects the air volume *simultaneously measured* in the volumeter connected to the occluded space of the external ear canal (31),
2 each change of volume within the closed middle ear is a function of a simultaneously *released* change of pressure, and vice versa,
3 a likewise constant and linear relationship between pressure and volume exists within the occluded space of the external ear canal as well as between the two adjacent closed spaces of middle ear and external ear canal

These cornerstones of the current volumetric methods are based on the indispensable pre requisite that the tympanic membrane can serve as a reliable *mediator* at the indirect recordings, thus allowing of computations according to Boyle's general gas law for the transfer of all local pressure changes to volume changes and vice versa

The intact tympanic membrane is thus being sed as a *mediator* at the indirect recordings for five different purposes

1 for the preparatory deviation from its "neutral" position
2 for its likewise preparatory and simultaneous change of the closed middle ear volume (both these effects are intentionally wanted *by the use of the pre-elected pressure* level)
3 for the recorded step by step return to its "neutral position (by the use of voluntary deglutitions)
4 for its causing simultaneous changes of pressure and volume also to be released in the occluded space of the external ear canal, *from which latter space all the quantitative volumetric measurements are recorded*, and
5 for the ultimate conclusions that the measurements recorded from the external ear canal are *reflecting the air flow through the* tubal lumen

The use of the intact tympanic membrane as a reliable mediator decisively rests on the pre requisite that it changes its position *only* and *passively* in response to pressure changes in the closed middle ear *This, however is not the case* Contrarily, the tensor tympani muscle moves the tympanic membrane *actively* by contractions and relaxations at all pressure changes during the quantitative recordings of volume in the current methods Even the tonus change of the tensor tympani muscle may influence the *volumetric* effect of the pressure changes in the closed middle ear, and to an extent that so far has not been *quantitatively* assessed A small pressure change may conceivably *not* cause any volume change at all if the tensor tympani muscle is able to keep the tympanic membrane in its ' neutral ' position

The significance of the tensor tympani muscle activity has not at all been taken into account in the current volumetric methods Boyle's general gas law is in reality non applicable for relevant mathematical transfers of volume to pressure, and vice versa The ΔV_{tm} is definitely no function of the ΔP_m

It may finally be stressed that the current volumetric methods (as well as the impedance and tympanometry *methods*) necessarily cause *non physiologic* tubal and middle ear conditions per se Thus, these methods cannot clarify the *physiologic* behavior of the tubal ventilatory mechanism *or* decode concerning the controversial views on the *physiologically permanently or only intermittently open* tubal lumen *or* reflect the physiologic and non physiologic activity of the tensor tympani muscle *Quantitatively, they cannot* assess the physiologic variability of the tubal patency Consequently the lack of physiological *normative data* for comparison jeopardizes the volumetrically recorded *non physiologic* measurements *at their clinical evaluation*

PART III

The Pre-requisites of the New Volumetric Methods

General and specific purposes of the volumetric methods

The new volumetric methods of recording the Eustachian tube function are designed for research studies and clinical tests The new arrangements of approach and instrumental equipment are first of all aimed at scientifically reliable and reproducable recordings of

1 the physiologic *behavior* of the tubal ventilatory mechanism,
2 the *quantitative* assessment of physiologic and non physiologic pressure and volume changes in the clinically healthy and diseased ears,
3 the locally *volume influencing* activity of the tensor tympani muscle, and
4 the relevant *significance* of the size of the mastoid pneumatization

When used for research studies the new volumetric methods comply strictly with the following general and specific rules

Avoidance of systematic errors

1 The tympanic membrane is not used for a *mediator* at the *indirect* quantitative recording of pressure and volume changes in the closed middle ear
2 Boyle s general gas law non applicable for relevant measures, is not used for computations converting actually measured pressure changes to corresponding volume changes or vice versa

Statistical controls

First of all, sufficiently large groups of individuals (respectively single ears) are required for the research studies Secondly, the range of variation of tubal patency, figures on average and distribution as well as random errors and significance of differences have to be computed and also produced in graphs Inter individual and intra individual differences (the latter informing on asymmetry and correlation coefficient between the two ears in the same individual) should form the basis for all clinical evaluation of results achieved

The pressure and volume measurements may be demarcated step by step *or* in a total sum comprising all measurements during a study *or* during a certain lapse of time (or even being related to some other demarcating unit) Each regular or additional voluntary deglutition also has to be demarcated although clinically unsuitable for prognostic evaluations

Physiologic tubal patency

Physiologic pressure and volume changes (as presumed to occur as a result of the continuous gas resorption in the clinically healthy middle ear) are subjectively non observable If they are quantitatively measurable, their respective range of variation has not been assessed with respect to inter- and intra individual differences

Selection factors for relevant research studies

Comparisons have to be made between

1 normal material (i e representative of the population),
2 several separate groups, each of which comprising a disease entity or a non-physiologic condition (in both alternatives *either* of the tube alone, *or* of the middle ear alone *or* of both) Further subgroupings may have to be made according to sex and age

Subgrouping according to healthy and diseased ears

When aiming to calculate relative risk figures for *development* of various tubal and middle ear diseases, as well as for clinical evaluation of measurements on varying tubal patency for *prognostic* purposes, comparisons have to be made between

1 one group anamnestically not having suffered from any middle ear disease (thus not representative of the population and not a normal material from a statistical point of view),
2 several separate groups, each of which comprising a disease entity or a non physiologic condition (in both alternatives *either* of the tube alone, *or* of the middle ear alone, *or* of both)

This specific grouping has been used in corresponding studies concerning the significance of the varying size of the mastoid pneumatization (25, 37, 74) It may be indicated to discuss these quantitative problems of mastoid pneumatization and tubal patency at a somewhat greater length

The degree of tubal patency as quantitatively recorded has been used for *prognostic* conclusions on healing tendency in modern otosurgery (8, 45, 49, 50, 84, 85) Comparisons concerning the post operative healing tendency have also been made between the varying tubal patency and the varying mastoid pneumatization (26, 51, 83)

As for *prognostic* conclusions from the assessment of the size of the mastoid pneumatization (planimetrically measured) (25, 77), they originally concern the development and non-development of

1 acute otitis media
2 central, respectively
3 marginal perforation of the tympanic membrane, and finally
4 secretory middle ear affection

and also lead to an indirect and limited conclusion concerning the *spontaneous* healing of central perforations (above all traumatic ones) The post-operative *healing* tendency was *not* related to size of mastoid pneumatization In fact, *comparisons* between the prognostic *post-operative* healing tendencies of respectively mastoid pneumatization and tubal patency are still lacking scientifically satisfactory support from sufficiently large groups Even the experience and skill of the surgeon is of importance (55)

Consequences of the zero border of tubal patency

The non physiologic tubal patency obviously (and the physiologic one presumedly) implies the intermittent return to the zero border (and is sometimes even caused from the outside (44)) Consequently, indirect measurings of varying tubal patency (in research studies) must include measurements of

a) the time to overcome the zero border situation, i e the time lapse between the start of the deglutition and the start of the air passage through the tubal lumen,
b) the air flow *speed* (possibly changing from one tubal opening occasion to another), and
c) the time of the tubal lumen to remain open (2, 3)

Presumably there is going to be a step by step decrease or increase in the volume of air passing the tubal lumen, which is dependent on these factors even though the local change of pressure may play the most important role Thus, even when disregarding the individually varying "strength" of the deglutition muscles themselves, each single measurement can be expected to show a consecutive change until final adaptation to ambient atmospheric pressure

The sensitivity of the quantitatively recording methods

The arrangements of approach and instrumental equipment for the recording must allow for measurements within the total range of variation These demands can be approximated by the use of some already available (even if not

scientifically satisfactory) figures (27, 31, 34, 36, 61)

a) The physiologic need per minute of substituting air

Deglutitions are reported (34, 36) to occur regularly once every minute when awake and once every five minutes when asleep This would imply approximately 1000 deglutitions during a 24 hour period Using this figure together with the measurements recorded by the current volumetric methods under non physiologic local conditions (27, 31) the need of 1–2 ml substituting air during a 24 hour period under local physiologic conditions has been estimated

In turn, the need *per minute* of physiologically substituting air of about 1–2 microliter (0 001–0 002 ml) would be indicated, and being equal to the volume of gas resorption in the closed middle ear during about one minute, i e between two regular and consecutive deglutitions (both of which actually alters the tubal lumen from its ' closed ' "resting position ' into its "open ' "working position ' during about 0 12–0 6 sec)

The actual size of one microliter (0 001 ml) *may be visualized by the comparison* with a grain of rice with its volume of about 25 microliter (0 025 ml) The air volume passing through the tubal lumen at each tuba opening deglutition would thus correspond to approximately one twentyfifth (1/25) of the volume of a grain of rice [1]

[1] As an alternative a physiologically *permanently open* tubal lumen may be using *breathing* (13 23) for the re aeration of the middle ear air spaces Then the calculations have to be based on the breathing frequency of about 15 *per minute* Each breathing would thus allow a re aeration with one fifteenth (1/15) of a microliter corresponding to about 1/375 of a grain of rice (Were each exhaling and inhaling phase re aerating the middle ear the *volume would correspond to 1/750 of a grain of rice*)

It may be added that the *physiologic* tubal lumen is maintained by some authors to remain permanently open (10 11 12) and by others to be occluded by a moisture mucus film (13 34 52 53 63–65) This would correspond to the breathing activity at the re aerating of the nasal sinuses (In turn it would also indicate that the regular deglutitions are serving only to remove the continuous saliva secretion)

b) The non physiologic need for substituting air

The diameter of the tympanic membrane may be estimated to approximately 10 mm The pre elected pressure level in a study (imposed in the middle ear) may be presumed to deviate the tympanic membrane about 3 mm from its "neutral ' position This would correspond to the shape of a cone with a volume of about 75 microliter (0 075 ml) thus corresponding to the volume of three grains of rice

Consequently, irrespective of the need for 1 or 3 or 5 or even 10 consecutive and always tuba opening deglutitions for the re adaptation to the atmospheric pressure (5), the *volume* recorder has to be sensitive for measuring all volumes from parts of a microliter up to 75 microliters

The change of one microliter air in the closed middle ear cannot be expected to cause an otoscopically determinable change of the tympanic membrane position from or to its "neutral ' position *Thus the ' completion of a study is unlikely to be assessed in this way* Furthermore, the "neutral ' position of the tympanic membrane does not necessarily indicate the same pressure (or even gas composition) on both sides of the tympanic membrane (61)

It is also important to stress the fact that the *physiologic* pressure and volume changes (i e in the clinically healthy ear) are subjectively non observable In daily life this is in contrast to the experiences caused by corresponding non physiologic disturbances They become subjectively evident as soon as the tubal lumen remains shut for a slightly prolonged lapse of time and occur repeatedly when suffering from a common cold The tubal lumen becomes increasingly incapable of keeping step with the continuous gas resorption in the closed middle ear Furthermore, fast atmospheric pressure changes during air flights promptly and disturbingly obstruct the tubal ventilatory mechanism even with voluntary efforts to adapt the pressure in the closed middle ear to that of the

surrounding atmosphere for instance by re peated deglutitions or yawnings

Muscles involved in the deglutition activity

The tuba opening effect of deglutitions (regu lar as well as additional voluntary ones) are maintained to be due to the activity of several pharyngeal muscles (14 16 17 23 24) The actual interplay between the tensor veli palati and the levator veli palati is still disputed The salpingopharyngeus muscle with its direct insertion on the tubal wall is sometimes observed (13 81) to be non intermingling with other pharyngeal muscles In fact profes sional marine divers learn to keep the tubal lumen constantly open during descents with out voluntary or repeated deglutitions (35) (It can only be guessed that this can be due to the exclusive use of the salpingopharyngeal muscle)

Synergism between the tensor palati and *tensor tympani muscles*

The synergism of the tensor palati and tensor tympani muscles has been substantiated in several studies (15–18 20 38 40 54 62) First of all they are anatomically shown to belong to the same muscle group by sharing a common insertion and having intermingling muscle fi bers Furthermore nerve fibers have been shown to connect their respective otic and glossopharyngeal ganglion Synergetic reflex activity has electromyographically been shown in the tensor tympani at voluntary swal lowings both in animal experiments and at cholesteatoma operations in human ears Although not tested the other way around the possibility of a reflex activity elicited from the tensor tympani muscle to the tensor pharyngeal muscles may at least be conceiva ble The tuba opening effect of the pharyngeal muscles may thus be conceived to be initiated from the tensor tympani muscle as well

The trigger factors of the tensor tympani muscle

The tensor tympani muscle has been shown (2–4 12 19 20 40 46 54 62) to be sensitive to minute changes of pressure in the external ear canal as well as to tactile stimulation from the eye and external ear regions However in contrast to the stapedius muscle *in man* and to both the middle ear muscles *in animals* the human tensor tympani muscle is non sensitive to *sound* stimulation (18) (except for taking part in a general startle reaction at sudden and intense noise) Although the new volumetric methods are not coping with reflex activities of the tensor tympani muscle the possibility of some *direct* trigger factor may be suggested If for instance minute changes of pressure or gas composition in the clinically healthy mid dle ear were influencing this would in any case conform to the findings and conclusions (50) stating that a number of nerve fibers ending epilemmely on the muscle and in the connective tissue between the fibers were as sumed to be sensory fibers On the other hand the repeated finding in human cholesteatoma ears (72) of a widely and per manently open tubal lumen (with its subjec tively disturbing symptomatology) does not rule out the possibility of a basic etiology *in* the growing cholesteatoma itself A spastic and permanent activity may be released via the tensor tympani muscle to the tuba opening pharyngeal muscles

Ciliary transport of mucus in the middle ear

The ciliary activity in the middle ear causes a transport of local mucus through the tubal lu men towards its pharyngeal orifice (63 64 65) Its direction is thus reverse to the physiologi cally always aspirated re aeration The sig nificance of these two opposite flows is still unknown as are most of the *physiologic* behavior of the ventilatory mechanism of the Eustachian tube

The "instant picture" characteristic of the tubal patency

Basically, all relevant quantitative measurings of tubal patency depend not only on the method of recording itself but also on casual changes of the tested subject. In clinically healthy as well as in diseased middle ears even a slight upper respiratory infection may influence considerably the tubal patency and thus the results of the measuring. This "instant picture" characteristic is more pronounced in diseased middle ears. Furthermore, in an ear presenting a central perforation of the tympanic membrane, the causing disease process may conceivably have changed the tubal patency, reversibly or permanently. The measurements recorded are thus of a restricted value and have to be clinically evaluated accordingly, especially when aiming at *prognostic* information.

Furthermore, in a case for a transplant closure of a central perforation of the tympanic membrane, the pre operatively and quantitatively recorded tubal patency presents an additional factor of insecurity. Thus, beyond the lack of *normative data* on physiology for comparisons and beyond the methodical inexactness, the tensor tympani muscle cannot be expected until *after* the successful healing to regain its active influence on the position of the tympanic membrane and its re-establishment of the reflex interaction with the tuba opening pharyngeal muscles (see also page 18).

In a clinically healthy ear the risk of "instability"[1] of the tubal patency is obviously less than in a diseased ear, and consequently can be presumed to give more reliable quantitative measurements. In turn, several factors of the *recording methods* have to be dealt with. First, only *indirect* measurings can be made, since the mere insertion of a catheter or a surgical instrument into the tube or the closed middle ear causes local non physiologic conditions. Even a myringotomy or the puncture of a mastoid air cell changes the local physiology. Moreover, all such *direct* recordings, especially protracted ones (even when disregarding the risk of an immediate bleeding *into* the communicating air spaces) may be causing reactive processes locally.

This rather pessimistic view on the acquiring of information on tubal patency as being *prognostically* useful in modern otosurgery may to some extent become somewhat more optimistic when having access to *normative data* on relevant physiology for orientation and indispensable comparisons at the clinical evaluation of the tubal patency actually recorded.

[1] In this connection it may be added that the obvious "instability" of the tubal patency in diseased ears stands in direct contrast to the "stability" of the size of the mastoid pneumatization (in adults). Even a long standing otitis in the mastoid air cells, a so-called chronic mastoiditis, has been shown *not* to decrease the size of the mastoid pneumatization (though of course influencing its content of air) (73). Even though planimetric measurements (25) have been found to strictly concur with the volumetric ones (30, 80), the *size* has only been claimed to be an *indicator* for as yet unknown biologic factors of growth (mostly genetic ones). They may conceivably also be responsible for the development and non-development of the various forms of middle ear diseases.

PART IV

Five New Methods for the Recording of the Eustachian Tube Function

SPECIFIC AIMS OF THE NEW RECORDING METHODS

Five new methods of volumetric recording have been designed for studies of the tubal ventilatory function They vary with the specific aims as for suitable arrangements of approach and instrumental equipment First, due attention has been payed to aims at (*a*) research studies, and (*b*) clinical tests The former are more intricate and laborious When comprising experimental surgery they have to be performed in animals (monkeys or other mammals) Clinical tests are designed to be simple and to avoid all unnecessary discomfort for the patient

The clinical tests for *prognostic* conclusions differ according to their use in (*a*) modern otosurgery, and (*b*) aviation and marine diving The former are used for testing ears with a tympanic membrane perforation and the latter for ears with an intact tympanic membrane Each alternative presents its own complex of mutually entangled volumetric problems

NEW INSTRUMENTAL EQUIPMENT

With some decisive alterations the quantitatively recording instruments used in the current volumetric methods constitute the basis for the new methods

A The original catheter (or tube) with its inflatable rubber cuff device (Fig 2) forks into two tubular branches at its distal end (Fig 8*a*) One of these branch tubes is occluded at its distal opening by a thin rubber membrane (Its elastic properties have to be assessed by the method used for the tympanic membrane system" in the current volumetric studies (7, 30, 33, 41, 80))

A tap device is also designed and inserted in the undivided part of the catheter (Fig 8*a*)

B The original puncturing mandrin needle is altered accordingly (Fig 9*a*)

C A set of steel containers are designed to be connected with the tap device of the catheter (respectively of the puncturing mandrin needle) and sized 1 ml, 2 ml, 3 ml, 5 ml and 10 ml (Fig 10, 8*b* and 9*b*)

THE PURPOSE OF THE INNOVATIONS

The divided catheter is to be used in all research studies as well as in the clinical tests At the quantitative recordings the occluded tubular branch is coupled to the *volumeter* and the other one to the *manometer* Thus both measurings can be made *simultaneously* since both measuring systems are representing closed recorders (the rubber membrane acting as a mediator towards the volumeter, *not* allowing the enclosed air itself to pass *into* the recorder)

The air containers can be used in every quantitative recording (Fig 8*b* and 9*b*) Each container can be consecutively coupled and thus adds its volume to that of the enclosed air space The significance of the volume increase can thus be disclosed in any *single* ear Even the size of the actual mastoid pneumatization has to be measured its planimetric size computed into volumetric size (30 80) but without any need for testing ears with all sizes of mastoid air cell system

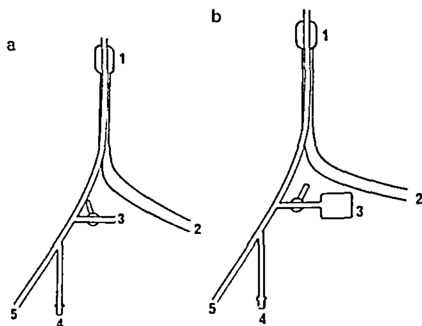


Fig 8a and 8b New device of rubber cuff system (allowing of simultaneous recordings of volume and pressure changes together with the disclosure of the importance of air content in the varying mastoid air cell system) 1 Rubber cuff 2 tube for glycerine inflation 3 tube for being connected to the air container set (8*a* with the tap closed and 8*b* with the tap open and a container connected) 4 tube of air flow meter device (with its outer orifice occluded by a thin rubber membrane) 5 tube to the manometer (outer orifice open)

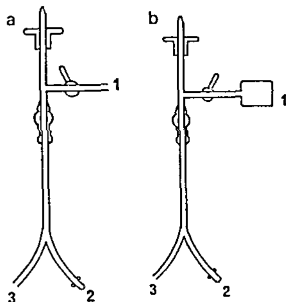


Fig 9a and 9b New device of puncturing mandrin needle 1 Tube for being connected to the air container set (9*a* with the tap closed and 9*b* with the tap open and an air container connected) 2 tube to the air flow meter device (with its outer orifice occluded by a thin rubber membrane) 3 tube to the manometer (outer orifice open)

NEW ARRANGEMENTS OF APPROACH

A new *mediator* for the *indirect* quantitatively recordings of pressure and volume changes is created by a *fenestration operation* of a terminal mastoid air cell (previously located roentgenologically) The uncovering of its lining

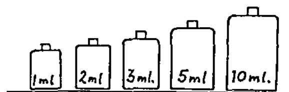


Fig 10 Air container set Note the possibility of using the air container set for the asessment of the influence of the varying size of the mastoid air cell system is dependent on the presumtion that the *varying air volume* is the influencing factor Diamant (1940) claims to be using the size of the mastoid air cell system *only as an indicator* of biologic factors determining the final size and the development of certain forms of otitis media as for instance acute otitis media with or without central drum perforation as well as marginal drum perforation with or without demonstrable cholesteatoma

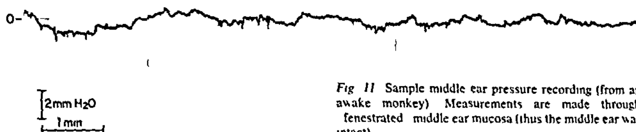


Fig 11 Sample middle ear pressure recording (from an awake monkey) Measurements are made through fenestrated middle ear mucosa (thus the middle ear was intact)

mucosa offers a mediator that is movable and *only passively* deviated by the pressure changes in the closed middle ear air space

The fenestration operation

The operation implies the free exposure of the mucous membrane lining of a terminal mastoid air cell

An incision is made through the galea and the periost These tissues are pushed aside, thus uncovering the cortex of the mastoid bone A dental burr is used parallel to the bone surface, gradually removing the cortex without rupturing the soft mucous membrane The mucous membrane is then used for a mediator at the indirect and quantitative measurings of all changes of pressure *and* volume within the communicating spaces of the middle ear

The mucous membrane is indeed very movable and *only* passively deviated by the pressure and volume changes It is not *directly* or *actively* deviated by the tensor tympani in contrast to the intact tympanic membrane used for a corresponding mediator in the current volumetric methods [1]

THE DISCLOSURE OF THE VOLUME INFLUENCING ACTIVITY OF THE TENSOR TYMPANI MUSCLE

The method implies double recordings *a*) from the fenestrated area and *b*) from the external ear canal (the latter in full accordance with the current volumetric except for the use of the two branched catheter (Fig 8*a*))

In these composite recordings all changes of pressure and volume can still be measured *simultaneously*, thus allowing of comparisons in six different constellations (see page 28)

[1] The fenestrated mucous membrane was used as a *mediator* at *indirect* and *protracted* recording of the *physiologic pressure changes in the clinically healthy ear* (Fig 11) The results are reported as follows (72) Note the absence of obvious stepwise changes in pressure (due to deglutition) Note further that pressure attains positive values and that deviations are restricted to small values These observations are typical of those made during six hours sessions over a period of seven days It would thus seem that under ordinary conditions middle ear pressure *deviates little and that it does not attain* the values commonly imposed on the middle ear in Eustachian tube function tests The use of high pressure values in such tests may lead to erroneous results and hence invalid conclusions regarding the state of function of the Eustachian tube

The *First* New Method

The *fenestration* method (Fig. 12)

Object

Research studies in animals of the *clinically healthy* ear (i e an ear with an *otoscopically* intact tympanic membrane and a *presumed* physiologic function of the tubal ventilatory mechanism)

Type of recording

All local pressure and volume changes are *simultaneously* and *indirectly* recorded from the fenestrated area

a) in alternative 1 under local *physiological* conditions (no use of the pressure chamber), and
b) in alternative 2 under *imposed* local *non-physiological* conditions (by use of the pressure chamber)

Surgery

Fenestration of a terminal mastoid air cell (previously located roentgenologically)

Instruments

1 The two-branched catheter (Fig 8*a*)
2 the set of air containers (Figs 10 and 8*b*),
3 common surgical instruments for the fenestration operation, and
4 the pressure chamber (for alternative 2)

The varying purposes of first new method

Alternative 1 is to be used to settle

A The still unsolved qualitative problems

a) as to whether the tubal lumen is *physiologically* open constantly or only intermittently
b) as to whether the *physiologic* re-aeration of the middle ear is using deglutitions or breathing (in- or exhaling or both)
c) as to whether the size of the mastoid pneumatization is *physiologically* of significance for the middle ear ventilation

B The still unreliable quantitative problems

a) as to whether the *physiologically* continuous gas resorption in the middle ear causes measurable pressure and volume changes (and thus basically being a test on sensitiveness of the measuring recorders),
b) as to whether the relationship between the *physiologic* pressure and volume changes is a throughout constant and linear one

Alternative 2 is to be used for reliable quantitative measurements of *non physiologic* changes of pressure and volume per se as well as their mutual relationships

Management

After successful fenestration the two-branched catheter (Fig 13) is air tightly sealed

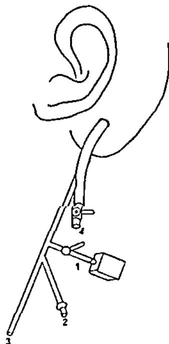


Fig 12 First new recording method fenestration of the terminal mastoid air cell New catheter with rubber cuff device 1 Tube for the air container set with air container in site 2 tube for the air flow meter device (its outer orifice occluded by a thin rubber membrane) 3 tube for the manometer (outer orifice non occluded) 4, tube for glycerine inflation

over the fenestrated area and its surrounding cortex of the mastoid bone

The occluded branch of the catheter is then connected to the air flow meter device, and the other branch to the manometer All quantitative measurings are made *simultaneously*, thus assessing the figures on pressure and volume changes as well as their mutual relationships

The new set of air containers can also be used consecutively during the study

When using the pressure chamber for measurings of *non physiologic* pressure and volume changes, the recordings have to be started already before the imposing of non physiologic pressure level elected The recordings continue until return of the original middle ear pressure

The *Second* New Method

The *puncturing* method (Fig. 13)

Object

Research studies in animals of the *clinically healthy* ear (i.e. an ear with an *otoscopically* intact tympanic membrane and a *presumed* physiologic function of the tubal ventilatory mechanism)

Type of recording

All local pressure and volume changes are *simultaneously* and *directly* recorded from the puncturing mandrin needle

a) in alternative 1 under local physiologic conditions (no use of the pressure chamber), and
b) in alternative 2 under *imposed* local *non-physiological* conditions (by use of the pressure chamber)

Surgery

The puncturing of a terminal mastoid air cell (previously located roentgenologically)

Instruments

1 The new two-branched puncturing mandrin needle (Fig 9*a*)
2 the new set of air containers (Figs 10 and 9*b*)
3 common surgical instruments, and
4 the pressure chamber (only to be used in alternative 2)

The puncturing of the mastoid

Using local anaesthesia the terminal mastoid air cell is punctured (precautions to be taken see below)

Management

For several reasons this puncturing method implies disadvantages common for all *direct* methods of relevant recordings Even when evading the risk of an immediate bleeding into the communicating air spaces, it must be remembered that the pressure on both sides of the intact tympanic membrane may be different *even physiologically* Thus, the procedure itself has to avoid an open communication *between the closed middle ear air spaces and the* ambient atmosphere with its possible resultant changing of the *physiologic* pressure in the mastoid air cell There are several precautions that can be taken in order to eliminate systematic errors of this kind (thus occurring *already prior to the start of the measurings)* So, for instance the puncturing mandrin needle must have also the branch for the manometer occluded during the puncturing Furthermore, the special lumen for the mandrin needle has

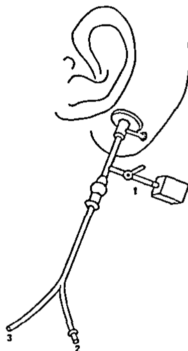


Fig 13 Second new recording method puncturing of the terminal mastoid air cell New puncturing mandrin needle 1 Tube for the air container with air container in site 2 tube for the air flow meter device (its outer orifice occluded by a thin rubber membrane) 3 tube for the manometer (outer orifice non-occluded)

to be sealed, and the removal of the mandrin must not be opening an airway between the middle ear and the ambient atmosphere Only with these precautions the initial differences of pressure on both sides of the intact tympanic membrane can be disclosed

Discussion on purpose

A relevant question is why the puncturing method should be used at all? It is true that the fenestration method, like the puncturing method, utilizes the mastoid air cell system in the quantitative recording of pressure and volume changes The difference is in fact the lack of knowledge concerning the initial physiology Nevertheless, the *direct* puncturing method can be used as a control on the *indirect* fenestration method just by disclosing differences initially occurring between middle ear and ambient atmosphere At the same time it may disclose the significance of a paracenthesis in a clinically healthy ear [1]

[1] It may be mentioned that the puncturing of the mastoid (for relevant *direct* recording) has been tried in two previous studies in about 20 human ears (27 31) However still a pre elected pressure level from the pressure chamber was used and imposed on the closed middle ear via the puncturing needle itself Thus non physiologic local conditions were still caused possibly also intervening with the physiologic behavior of the tubal ventilatory mechanism In one of the studies (31) however no pre elected pressure level was at all imposed during a first stage of the quantitative recording of volume changes But also in this study the intact tympanic membrane was serving as a mediator at the recordings from the occluded proximal space of the external ear canal Furthermore no *simultaneous* measurings of pressure and volume changes were made and the role played by the tensor tympani muscle was not considered Consequently for several reasons this *directly* recording study was not grammarly designed and the results do not give information on relevant quantitative physiology

The *Third* New Method

The double check indirect–indirect method (Fig. 14)

Object

Research studies in animals of the *clinically healthy* ear (i e an ear with an *otoscopically* intact tympanic membrane and a *presumed* physiologic function of the tubal ventilatory mechanism)

Type of double recording

All local pressure and volume changes are *simultaneously* recorded in double, i e

a) *indirect* from the fenestrated area (as in the *first* new method) (see page 23), and
b) *indirect* from the occluded external ear canal (as in the *current* volumetric methods (see page 11) but still with the two-branched catheter)

and again

in alternative 1 under local physiological conditions (no use of the pressure chamber), and
in alternative 2 under locally *imposed* non physiologic conditions (by use of the pressure chamber)

Instruments

1 The same equipment as in the *first* new method,
2 an additional set of pressure and volume recorders, and
3 an additional two-branched catheter

Surgery

The fenestration operation as described for the first new method

Purpose

The third new method aims exclusively to disclose the role played by the tensor tympani muscle under physiologic as well as under non physiologic local conditions

The tensor tympani muscle deviates *actively* and the local change of pressure *passively* the intact tympanic membrane from its ' neutral position Thus the muscle activity may imply a decrease or an increase of the local change of *volume* caused passively by the changing pressure

Management

Alternative 1 (local physiology)

The two-branched catheters are recording (*a*) from the fenestration area, and (*b*) from the external ear canal *simultaneously* They are connected to their respective full set of recorders always with the occluded catheter branch to

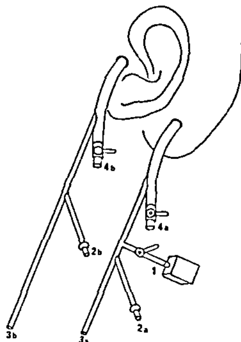


Fig 14 Third new recording method New rubber cuff device in duplex one placed over the fenestrated terminal mastoid air cell and the other in the external ear canal for the parallel recordings of simultaneously measured changes of volume and pressure 1 2 3 4 to be used as given in Figs 11 and 12

the volumeter and the other branch to the manometer

Alternative 2 (local non physiology)
After all four catheter branches have been connected as in the alternative 1, the measurings are started, thus prior to the imposing of the pre elected pressure on the 'closed' middle ear air spaces The changes of pressure and volume are thus recorded starting from the actual local physiology and then continued during non physiology and until the return to final re adaptation to ambient pressure

Discussion to the third (and the (next) fourth) new method

Each of the double check methods is quantitatively measuring all pressure and volume changes in the closed middle ear air spaces *simultaneously* Comparisons can thus be made in six different constellations

two comparisons of pressure and volume recordings *within* each set of recorders i e pressure to volume, and
four comparisons *between* the two sets of pressure and volume recorders, i e pressure to pressure, volume to volume pressure to volume and (reversed) volume to pressure

The *third* and the *fourth* methods may even be used for mutual controls At the same time all methods in the research studies may prove the necessity of measuring pressure and volume changes *simultaneously*

The *Fourth* New Method

The double check direct–indirect method (Fig 15)

Object

Same as the third new method

Type of double recording

All local pressure and volume changes are simultaneously recorded in double, i e

a) *direct* from the punctured mastoid air cell (as in the second new method (page 25)), and

b) *indirect* from the occluded external ear canal (as in the *current* volumetric methods (see fig 3) but still with the two-branched catheter), (precautions see page 25)

and again

in alternative 1 under local physiologic conditions (no use of the pressure chamber), and

in alternative 2 under locally imposed non-physiologic conditions (by use of the pressure chamber)

Instrumental equipment

1 The same equipment as in the *second* new method (page 25),

2 an additional set of pressure and volume recorders

Surgery

The puncturing of a terminal mastoid air cell (as described for the *second* new method, page 25)

Management

The same as in the *third* new method except for special precautions to be taken at the puncturing itself of the mastoid air cell

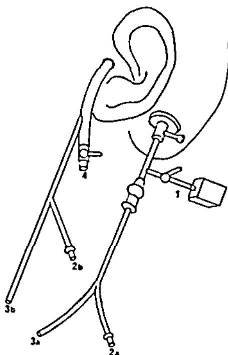


Fig 15 Fourth new recording method New puncturing mandrin needle (in a terminal mastoid air cell) and new rubber cuff system in the external ear canal for the parallel recordings of simultaneously measured changes of volume and pressure 1 2 3 and 4 to be used as given in Figs 11 and 12

The *Fifth* New Method

The clinical tests (Fig 16)

Object

Clinically healthy as well as secretory middle ears *with an intact tympanic membrane*, respectively diseased ears *with an open tympanic membrane perforation*

Type of recording

All local pressure and volume changes are *simultaneously* recorded

in closed ears *indirectly* (by using the intact tympanic membrane for a mediator), and

in open ears *directly* (passing through the tympanic membrane perforation)

In all tests the measurements are recorded from the occluded part of the external ear canal, starting *before* imposing pressure from the pressure chamber and then continuing up to some pre selected pressure level and back to ambient atmosphere pressure

Surgery

None

Instruments

1 The new two-branched catheter (Fig 8*a*)
2 the new set of air containers (Figs 10 and 8*b*),
3 the pressure chamber for all tests and
4 the pressure and volume recorders of the *current* volumetric methods

Purpose

a) In the clinically healthy ears the tests give information on the fitness of a prospective professional aviator or marine diver candidate,
b) in the secretory middle ear affections they inform on the effect of different non operative therapeutic measures and
c) in diseased ears with a tympanic membrane perforation they may inform on the chances of a postoperative healing in cases of myringoplasty

Management

The proximal end of the two branched catheter is lodged in the bony part of the external ear canal and its rubber cuff device is inflated The branch occluded with the rubber membrane is connected to the volumeter, and the other branch to the manometer Thus the air spaces of the middle ear, the mastoid pneumatization, the occluded part of the external ear canal and the catheter are communicating and occluded in both connections allowing of simultaneous measurings of pressure and volume changes during the study

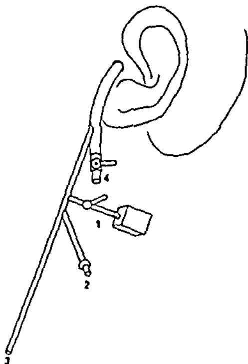


Fig 16 Fifth new recording method for *clinical tests* New rubber cuff device lodged in the external ear canal 1 2 3 and 4 to be used as given in Figs 14 and 15 for the recording from the external ear canal

Discussion

All clinical test measurements have to be evaluated at comparisons with *normative data* on physiology In closed ears using the intact tympanic membrane for a mediator at the quantitative recordings, the role played by the active tensor tympani muscle must also be taken into account

The common "instability" of the tubal air passage capacity, especially pronounced in "open" ears, is basically jeopardizing all results from the clinical tests (see page 19) Furthermore, in "open" ears the *prognostic* information wanted has to be found in testing the tubal patency *pre-operatively* whilst the information wanted concerns the tubal patency *after* the successful healing of the tympanoplasty operation Thus for the time being only retrospective *comparisons* between the pre-operative measurements and those recorded post-operatively can support future reliable *prognostic conclusions* i e when such comparisons are available for statistical computations of relative risk figures

So far, it is in fact only known for certain that a complete and permanently closed tubal lumen is a definitely "bad" sign Still it is only taken for granted

a) that any degree of tubal patency is sufficient for *some* chance of post-operative healing,
b) that some "lowest" limit of tubal patency informs of no chance of post operative healing, and
c) that a "better" tubal patency is a prognostically "good" sign (i e without so far giving reliable figures for the nomination of "better" and "good")

Very important is also the selection factors in the studies of tubal patency So, for instance, in a group selected for transplant operations, cases of central and marginal tympanic membrane perforations cannot be grouped together The post-operative results have to be differing between these two diseases, since they differ etiologically, clinically and therapeutically Still it is common to bring these two diseases into one group, lately also adding the secretory middle ear affection to the *misnomer* of "chronic otitis" (78) This seems to be common not only when studying the tubal patency but also in all relevant problems of otology A strict selection factor is essential for the grouping of diseases when aiming at reproducable results of for instance therapy

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OTO-LARYNGOLOGICA

SUPPLEMENT 349

VESTIBULAR DISORDERS IN MEDICALLY MANAGED CHRONIC RENAL INSUFFICIENCY

By

Kyösti Laitakari

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SUPPLEMENT 349

VESTIBULAR DISORDERS IN MEDICALLY MANAGED CHRONIC RENAL INSUFFICIENCY

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Turku 1977

Turku 1977 Kirjapaino Pika Oy

To My Wife

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1 INTRODUCTION

The last few decades have brought new possibilites to lengthen the life span of the patients suffering from chronic renal insufficiency This has aroused growing interest in the numerous complications of uraemia among others in hearing and balance disorders There are some recent reports on hearing loss in patients undergoing haemodialysis and transplantation therapy In spite of frequent complaints of vertigo and dizziness no nystagmography studies dealing with renal failure patients (excluding a report on Alport s syndrome) have yet been published

II REVIEW OF THE LITERATURE

1 *Vestibular disturbances and chronic renal failure*

Grahe (1924) reported that 13 of his 32 nephritis patients studied without any electrical equipment had spontaneous nystagmus and/or a positive past pointing test Pathological responses to caloric or rotatory stimuli were observed in 18 patients

Beaney (1964) reported that only 5 patients of 262 with severe renal failure had vestibular disturbances and each was connected to ototoxic antibiotics

Half of the 105 uraemic patients of Yassin et al (1966) had some kind of vertigo or dizziness 5 rotatory vertigo with spontaneous nystagmus 20 rotatory vertigo without spontaneous nystagmus and 25 felt only zzy

n a later study of Yassin et al (1970*b*) 4 patients in advanced renal failure were xamined calorically without nystagmography 28 per cent had normal caloric responses 57 per cent hyperexcitable labyrinth and 15 per cent canal paresis Hyperexcitability was observed mostly in acute uraemia and in only 14 per cent of those suffering from chronic uraemia It was clearly parellel with hyponatremia Thirty seven per cent of the patients in this series complained of vertigo

Miller et al (1970) found vestibular hyporeactivity with nystagmography in all of their 4 tested cases of Alport s syndrome (hereditary nephropathy and deafness)

Wigand et al (1972) examined calorically using Frenzel s glasses 16 of their later mentioned 60 patients In two cases the reactivity was notably diminished but no hyperreactions or total loss of reactivity occurred

2 *Hearing loss and chronic renal failure*

There are some scattered earlier reports on hearing loss associated with renal failure (e g Gradenigo 1880, Wittmaack 1905) Grahe (1924) published a voluminous monograph dealing with 33 uraemic patients examined thoroughly but without any electroacoustic equipment Eighty two per cent of these nephritis patients had high tone hearing loss No morphologic changes were noted in the temporal bones of 18 patients examined histologically post mortem The cause of the hearing loss was assumed to be the central toxic effect of uraemia No correlation was established between vestibular disorders and hearing loss

Beaney (1964) studied 262 patients during management of severe renal failure and found only one sudden deafness and this was attributable to ototoxic antibiotics

Ransome et al (1966) reported that 12 of 60 patients with advanced renal failure had some degree of hearing loss 7 with slight high tone loss and others with more severe hearing loss Polybrene utilized in haemodialysis was then mistakenly blamed as a causal agent

Eight of 105 patients beginning dialysis treatment were noticed to have perceptive deafness of varying degree by Yassin et al (1966) Hearing loss tended to improve to

some extent with dialysis indicating that there are some uraemic toxins thought to be causative agents which could be removed by dialysis In a later study Yassin et al (1970*a*) examined 71 patients with terminal uraemia with an audiometer and observed a high rate of hearing loss moderate to severe deafness occurring in 75 per cent Hearing loss seemed to correlate fairly well with hypo natremia but not with potassium nor haemoglobin nor with sex or age Im provement in hearing was reached only if the sodium balance was corrected

This opinion of hyponatremia as the causative agent was supported by Wigand et al (1972) They studied audiometrically 60 patients with advanced renal insufficiency After individual age and sex correction they found 15 – 20 dB hearing loss up to 2 kHz and 20 – 30 dB high tone loss Hyponatre mia and glomerular filtration rate corre lated best to hearing loss at 8 kHz Pure tone and speech audiometry allowed no certain localization but gave an impression of a primarily cochlear lesion especially in the absence of other neurological disorders The duration of renal failure was felt to be im portant

Kopsa et al (1972) investigated 20 patients with advanced uraemia with pure tone audiometry and the SISI test Some degree of high tone loss was observed in almost all patients The SISI scores were mostly over 50 per cent and in 8 patients exceeded 80 per cent Hearing loss was therefore said to be localized in the cochlear hair cells The authors felt that hyponat remia was not important as a causative factor

The effect of renal transplantation on uraemic hearing loss was studied by Mitschke et al (1973 1974 and 1975) An improvement of 8 – 30 dB six months after successful transplantation was perceived in ten patients In half of the cases the SISI test changed from positive (over 50 per cent) to negative No correlation was established between hearing loss and hypertension or serum electrolytes

Bergstrom et al (1973) tested 224 patients in terminal uraemia audiologically finding 91 cases of sensorineural hearing loss Only 11 per cent remained unexplained by factors other than renal disease e g noise exposure heredity and ototoxic medication A histo pathological examination was performed post mortem in ten cases but no consistency was found in the pathological changes of the temporal bones

Johnson & Mathog (1976) and Johnson et al (1976) demonstrated significant high tone hearing loss and marked fluctuations in 71 patients undergoing haemodialysis treatment The losses were independet of serum Na K Ca urea nitrogen creatinine glucose and blood pressure Most apparent were hearing losses at 6 4 and 8 kHz in this order No progression was noticed with age It was not possible to determine whether the hearing loss was presbyacusis accentuated by unknown factors in the renal patient

A group of 602 patients suffering from terminal renal failure was audiologically examined by Quick (1976) He detected 107 cases with hearing loss It was possible to identify factors other than uraemia contri buting to hearing loss in the majority of cases Quick (1976) got the impression that the hearing loss is rarely due to one cause but stems from the interaction of many factors

Thomsen et al (1976) studied 281 patients under haemodialysis therapy finding hea ring or vestibular disorders in 26 patients These patients had had on the average more dialyses than the others More information of the methods used is needed for further evaluation of this study

Oda et al (1976) studied audiologically 290 haemodialysis and renal transplant patients and observed that 43 patients (14 8 per cent) developed significant hearing loss which could be directly attributed to the therapy of the kidney problem The cl[illegible]

pathological findings of 16 temporal bones of 8 patients were presented. All the transplant patients showed blue stained concretions in the stria vascularis and/or in the vestibular receptors. The changes noted in the 16 cochleas ranged from mild loss of outer hair cells and spiral ganglion cells to complete absence of the organ of Corti. The severity of the clinical and histopathological temporal bone findings was directly proportional to the number of haemodialyses and transplants to which the patient had been subjected.

Henrich et al. (1977) followed the hearing acuity of 20 patients under chronic haemodialysis therapy over a period of 1–4 years. Fourteen patients had some measurable (over 20 dB) hearing loss at the beginning of the study: 10 patients had mild and 4 had moderate sensorineural hearing defects. Fifteen patients showed unchanged hearing, 2 patients decreased and 3 patients improved hearing during the follow up period. The changes, though, were minute. The authors concluded that if some aspect of uraemia is in part responsible for the initial hearing loss observed in some of these patients, then the results suggest that this nerve damage occurs early in the course of uraemia or that adequate dialysis is successful in preventing further deterioration in a majority of patients.

3. Ototoxicity of streptomycin, kanamycin and furosemide. Streptomycin and kanamycin are antibiotics of the aminoglycoside group. All aminoglycosides are to some extent ototoxic and some of them also nephrotoxic (Erlanson & Lundgren 1964). The changes are probably located in the sensory cells of the inner ear. Aminoglycosides are excreted by kidneys. Renal insufficiency can thus potentiate the toxicity.

Streptomycin almost exclusively affects the organ of balance, producing symptoms of dizziness and usually also bilateral reduction or disappearance of the caloric reaction.

Kanamycin mainly causes cochlear damage which begins with high tone loss and may lead to rapid deterioration of the hearing (Kohonen 1965).

High doses of furosemide are known to cause transient hearing loss and vertigo after intravenous administration associated with renal failure (Wigand et al., 1972, Brown 1973, Cooperman & Rubin 1973). This is obviously caused by the effect of furosemide on the active electrolyte transport through sensory cell membranes in the inner ear.

III MATERIAL

1 *Patients* From the outpatients suffering from medically managed chronic renal insufficiency visiting the University Central Hospital of Turku successive 59 patients (31 females and 28 males) were selected fulfilling the following requirements no known ear or vestibular disease and serum creatinine level at least 200 mmol/l (2 3 mg/100 ml) One ear suffering from secretory otitis media (without previous knowledge) was excluded The ages varied from 17 to 71 years mean 50 2 and S D 14 6 (Fig 1)

2 *Controls* The reference group was built up to meet the age distribution of the patients It consisted of 13 females and 7 males whose ages varied from 21 to 68 years mean 50 3 and S D 14 0 (Fig 1) Males and females were balanced in age groups above 40 years The subjects were not allowed any knowledge of ear or vestibular diseases nor of any other neurological disorders The

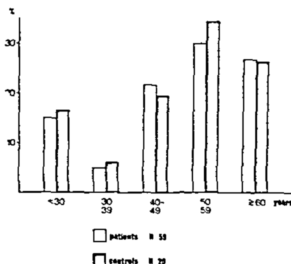


Fig 1 Age distributions

procedure was similar with this series expect that audiological evaluation was limited to pure tone audiogram No subjects were excluded on the basis of the test results

IV METHODS

1 *Case history* The following facts were gathered from the internist's chart diagnosis, duration of the renal insufficiency, accomplishing hypertension, blood haemoglobin and serum creatinine levels and medication given The subjects were asked about any previous or existing balance, neurological or hearing disorders Consumption of alcohol, nicotine, coffee and drugs not prescribed by the internist was recorded Aminoglycoside antibiotic therapy was noted in the case history

2 *Clinical examination* A thorough routine otological examination was performed The function of each cranial nerve was tested separately Muscular co ordination as a sign of cerebellar function was tested with finger to nose and pronation supination tests Standing walking and stepping on spot blindfolded were included in the program reflecting cerebellospinal and vestibulospinal functions Spontaneous and positioning nystagmus to both lateral positions and head hanging, including shaking the subject's head were observed in the dark with Frenzel's glasses

3 *Electronystagmographical (ENG) tests* All tests were performed in a darkened room employing an AC direct ink writing amplifier (Elema Mingograph 34) except in the rotatory tests where a built in AC amplifier (Polman Mod 11) was utilized The time constant used was 2 0 seconds, the upper limit was 70 Hz. Disposable ECG electrodes (No 737 by Simonsen & Weel) were fastened as near the outer canthi of eyes as possible, the reference electrode was secured on the forehead The electrical contact was improved by cleaning the skin with 70 per cent ethanol and by employing electrode paste

Complete darkness was attained by wearing welder's goggles lined on the inside with black paper in a carefully darkened room "Relaxed attention" (Torok, 1970) was maintained by speaking to the subjects and telling them to keep eyes open and to look straight forward No "mental arithmetics" were used as recommended by Torok (1970)

Calibration was performed by looking in turn at two illuminated spots on the wall at 2 5 meters distance from the subject, diverging by ten degrees The calibration task was carried out as often as possible to eliminate the error caused by fluctuations of corneoretinal potential (Aantaa, 1970)

In pendular eye tracking test (PETT) the pendulum had a frequency of 1/3 Hz Inability to produce at least one smooth sinus curve was considered pathological

Optokinetic nystagmus (OKN) was provoked by horizontally moving black stripes which diverged by 27 degrees They were projected on a screen which covered approximately 60 degrees of the central visual field Velocities of 20, 30, 40 and 50 °/s to the left and right in turn during 18 seconds were used in this sequence OKN was considered abnormal if a side difference existed without explaining spontaneous nystagmus

Spontaneous (SN) and positional nystagmus (PN) were recorded in complete darkness the eyes closed as well as open, in

supine and both lateral positions The effects of deep breathing and of holding the breath were noted

In the caloric tests the head was supported with a cushion at 30 degrees upward from supine Thermostatically regulated water of 30°C and 44°C at a flow rate of 3 ml/s was instilled in the ear canal over a period of 30 seconds Painlessness of the irrigation tube insertion was ensured by rounding the end of the plastic tube and dipping it into water soluble lubricating jelly The patient s eyes were kept open in total darkness

When the culmination phase was reached 70 seconds after the beginning of the irrigation the subject was told to fixate on a light spot in the ceiling The ocular fixation index (OFI) was regarded as pathological if a less than 50 per cent decrease in nystagmus amplitude or eye speed of the slow phase followed (Demanez & Ledoux 1970)

The maximal slow phase velocity was approximated by summing up the amplitudes within each 5 second period The irrigation sequence was 44°C left 44°C right 30°C left and 30°C right

Side difference canal paresis (CP) and directional preponderance (DP) were considered to exist if they exceeded 20 per cent Bilateral hyporeaction was noted if the summed maximal eye speed (44L+44R+30L+30R) did not reach 40 °/s

The rotatory tests were carried out with Polman s rotating chair Mod 11 e 111 which enables a smooth angular acceleration and deceleration from 0 2 $°/s^2$ to ca 8 $°/s^2$ The chair was calibrated both empty and with 72 kg mass separately for both acceleration and deceleration in both directions The following procedure was employed a basal constant angular velocity of ca 20 °/s was attained to avoid an initial jerk The time required for ten turns (t_1) was measured Then the time (t_2) needed for ten successive turns at a constant acceleration rate (α) was recorded Final velocity was then determined at a constant speed the resulting time (t_3) corresponding to ten rotations The formula $\alpha = 3600° \frac{t_1 - t_3}{t_1 t_2 t_3}$ gives the value of the acceleration rate At the range 0 2 $°/s^2$ — 1 5 $°/s^2$ the standard deviation (S D) of the measurements remained within 3 per cent reflecting the great accuracy of the chair

The nystagmus threshold for angular acceleration and deceleration was determined as follows At a basal velocity of 20 °/s an ENG was recorded to detect any spontaneous nystagmus Then a 0 2 $°/s^2$ acceleration was applied over a 90 second period followed by a 90 — 120 second period of constant velocity The subject was then decelerated at 0 2 $°/s^2$ during the final 90 seconds thus reattaining the initial velocity of ca 20 °/s After 90 — 120 seconds the procedure was repeated with 0 4 $°/s^2$ then 0 6 $°/s^2$ etc until detectable nystagmus was noticed The threshold values were controlled by rotating the subject in the other direction as well The lowest accelerations (decelerations) needed to produce nystagmus in each respective direction were taken as the two thresholds If spontaneous nystagmus existed the thresholds were excluded

The postrotatory test was performed by accelerating the chair from 0 °/s to 60 °/s at 1 0 $°/s^2$ then stopping it abruptly (1/3 s) thus creating a short maximal stimulation of 180 $°/s^2$ to the vestibular apparatus (Claussen 1975) The maximal eye velocity of the slow phase of nystagmus was measured over a 5 second period (Torok 1966)

To evaluate the ENG findings three groups were formed Group A (normal) — PETT and OKN normal and OFI normal in at least 3 of 4 caloric tests no CP or bilateral hyporeactivity Group B (central lesion) — PETT and/or OKN pathological and/or OFI abnormal in at least 2 of 4

caloric tests Group C (peripheral lesion) — CP or bilateral hyporeactivity

The PETT performance depends on the foveal (central) retinoocular tracts (Corvera et al, 1973) the OKN test on the fundal (peripheral) retinoocular tracts (Honrubia et al, 1968, Dix & Hood, 1971) and the OFI on the central inhibition of vestibular nystagmus requiring though an intact foveal retinoocular function (Demanez & Ledoux, 1970, Hart, 1973) Uni and bilateral hyporeactivity are the purest signs of a peripheral vestibular lesion including lesions of the first sensory neuron The hyperreaction to vestibular stimuli is probably due to a lack of central inhibition rather than to increased irritability of the end organ (Kornhuber, 1969) However, it was not used as a differentiating criterion because of a lack of information about its final nature and normal limits SN, PN and DP are so common in otherwise normal population (Coats, 1969, Barber & Wright 1973, Stroud, 1973) that they could not be used either Moreover the registered intensity of the nystagmus is highly dependent on the real direction of the nystagmus (oblique, rotatory nystagmus would have been overlooked)

4 *Audiological evaluation* Pure tone audiograms were taken by skilled examiners using Madsen OB 60 audiometers calibrated according to ISO R389, 1964 Air conduction thresholds were corrected with mean socioacusis in ten year periods according to Palva, A & Jokinen (1970) Speech reception thresholds and discrimination scores were determined (Palva, T, 1952) The threshold tone decay test was administered at 2 kHz beginning at 10 dB SL (sensation level) and lasting 3 minutes (Palva, T, 1964) Loudness recruitment was tested with Fowler's (1936) binaural loudness balance test when possible and in cases with symmetric hearing loss Reger's (1936) monaural test was administered The patients were examined for stapedius reflex threshold at 2 kHz (Metz, 1952) A Madsen Acoustic Impedance Bridge Mod ZO 70 and Beltone audiometer Mod 15 A were used

5 *Statistical methods* Age corrected audiograms were analysed with Student's t test The normality of the distributions of this testing was verified with probability paper Differences between standard deviations (S D) were tested with the F test Correlations were analysed with the linear correlation coefficient r and by comparing it to the significance table of r Age, creatinine and haemoglobine levels and the duration of renal disease in different groups were tested with Mann Whitney's U test Fourfold tables were calculated with Fisher's exact test The following symbols for significance levels were utilized $^{o} \hat{=} p \leq 0.1$, $* \hat{=} p \leq 0.05$, $** \hat{=} p \leq 0.01$, $*** \hat{=} p \leq 0.001$

V RESULTS

1 *Medical data* Serum creatinine levels varied from 200 to 1788 mmol/l (2 3 – 20 mg/100 ml), mean 489 and S D 357 (Fig 2)

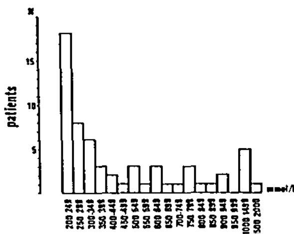


Fig 2 Serum creatinine levels of the patients (normally under 120 mmol/l)

Fig 3 Haemoglobin concentrations of the patients

Blood haemoglobin levels ranged from 65 g/l (6 5 mg/100 ml) to 162 g/l, mean 118 and S D 27 (Fig 3) Forty four patients (75 per cent) were being treated for arterial hypertension Duration of the renal insufficiency was often uncertain and varied from one year to several decades (Fig 4) The

Fig 4 Known duration of the renal failure

underlying disease was chronic pyelonephritis (treated with antimicrobics) in 31 patients, glomerulonephritis in 9, analgetic (phenacetine) nephropathy in 8 and other renal diseases in 20 patients Thirty-one patients were taking furosemide, 26 of them only 40—80 mg a day Seven patients were known to have had aminoglycoside antibiotic therapy in the past, 6 streptomycine and 1 kanamycine

2 *Results of the questioning* Tinnitus and/or hearing loss were complained of by 18 patients and by 4 subjects in the control series Eight[0] patients had rotatory vertigo every now and then which was not quite significantly more frequent than in the reference group (no vertigo)
Twenty one[**] patients complained of some kind of vertigo or dizziness none in the control series Six male and two female patients were cigarette smokers two men smoked in the control group

3 *Clinical examination* No clear abnormalities could be verified with the otolaryngological and neurological examination employed

Nystagmus after head shaking was observed with Frenzel s glasses in 17[**] renal patients and in none of the control subjects (p = 0 004) In 7 of these 17 cases no spontaneous or positional nystagmus were found in the ENG recording in 8 spontaneous and/or positional nystagmus occurred at least in some point of the recording to the same direction and in 2 only to the opposite direction

4 *ENG findings* Spontaneous or positional nystagmus was observed in 27 uraemic and in 6 control subjects The difference between the incidences was not significant p = 0 11

The proportion of B and C groups together (disorder of the vestibular system) in patients (40 of 59) was higher[***] than in reference subjects (5 of 20) p 0 0009 (Table I) Central lesions (B) (30 of 59) were more frequent[*] p = 0 03 and peripheral lesions (C) were clearly[*] over represented (14 of 59) in the uraemic series p = 0 0104 Four patients had both central (B) and peripheral (C) findings this group being too small for independent statistical evaluation The central lesions in the reference group consisted of four pathological PETTs (all over 50 years) and one asymmetrical OKN (28 years)

Somewhat higher[0] mean nystagmus thresholds for angular acceleration (0 28 °/s^2 in controls and 0 32 °/s^2 in patients) were seen in the patient series (Table II) In this series the S D of thresholds (0 21 °/s^2) was greater[*] than in the control series

Group	Patient	Reference
A (normal)	19	15
B (central lesion)	30	5
C (peripheral lesion)	14	0

Table I *The electronystagmographic findings*

	N	Mean	S D	Min	Max
Patients	98	0 32	0 21	0 2	1 4
Controls	40	0 28	0 10	0 2	0 4

p < 0 1

Table II *The nystagmus thresholds for angular acceleration (°/s^2) spontaneous nystagmus excluded N = number of thresholds*

(0 10 °/s2) Results of the postrotatory tests in each group (9 6 °/s in controls and 9 2 °/s in patients) were equal to each other (Table III)

The patients with and without furosemide medication were evenly distributed in various ENG groups (ABC), A 9 and 10, B 15 and 15, C 8 and 6, as were the patients with and without previous aminoglycoside antibiotic administration, A 3 and 16, B 3 and 27, C 1 and 13

Table IV shows the mean ages of A, B and C groups, which were 43 8, 56 0 and 50 6 years, respectively The patients with central vestibular lesions (B) were on the average older** than the patients of group A ("normal"), $p < 0\,01$ The difference between groups A and C was not significant

The mean known duration of renal insufficiency in groups A, B and C (7 3, 6 2 and 6 4 years respectively) did not differ significantly and the patients with less than and over 5 years duration were evenly distributed between the ENG groups (A 9 and 10, B 16 and 14, C 10 and 4)

The distribution of hypertensive (medicated) and normotensive patients between the ENG groups was even, A 15 and 4 B 20 and 10, C 11 and 3

The mean creatinine levels did not differ significantly between groups A B and C (575, 397 and 608 mmol/l, respectively) Dividing the patients according to serum creatinine level (under and over 350 mmol/l) did not help to uncover any significant differences between the groups (A 8 and 11, B 19 and 11, C 5 and 9)

The mean blood haemoglobin concentration in group A was 113, in B 123, in C 110 g/l (Table V) and the distribution of anaemic (< 110 g/l) patients and others in group A was 8 and 11, in B 9 and 21 and in C 6 and 8 The differences were not significant

	N	Mean	S D	Min	Max
Patients	104	9 2	4 7	0 9	20 0
Controls	40	9 6	4 9	1 3	20 0

Table III *Maximal postrotatory eye velocity (°/s) N = number of tests*

	N	Mean	S D	Min	Max
A ("normal")	19	43 8	15 2	20	69
B (central)	30	56 0	11 9	17	71
C (peripheral)	14	50 6	15 2	18	66

Table IV *ENG findings and the age (years) of the patients*

	N	Mean	S.D.	Min	Max
A ("normal")	19	113	29.5	65	156
B (central)	30	123	25.1	69	169
C (peripheral)	14	110	27.9	69	158

Table V. *ENG findings and mean haemoglobin level (g/l).*

5. *Audiological results.* The uraemic patients had significantly poorer mean hearing thresholds than the control persons at 2, 4, 6 and 8 kHz, the significance being greater the higher the frequency (Table VI, Fig. 5). All the included hearing impairments were of the sensorineural type.

Speech discrimination was 100 per cent in 98 of 117 ears, between 90 and 100 per cent in 18 ears and 80 per cent in one ear. The Fowler's or Reger's test showed full recruitment in 9 ears with the stapedius reflex threshold under 65 dB SL (sensation level) and in 4 ears without successful stapedius

		0.5	1	2	4	6	8 kHz
Patient group, 117 ears							
	Mean	1.5	2.0	5.1	6.0	11.6	9.8
	S.D.	8.7	10.2	14.3	17.2	18.9	18.1
	Min	*-12*	*-17*	*-26*	*-33*	*-35*	*-32*
	Max	28	39	51	47	57	54
Reference group, 40 ears							
	Mean	-0.3	0.1	-0.2	-2.6	1.6	-2.6
	S.D.	5.2	6.6	9.0	16.0	19.2	15.7
	Min	-7	-14	-16	-38	-40	-37
	Max	13	23	19	27	54	37
One-tailed $p <$		0.15	0.15	0.025	0.005	0.0025	0.0005

Table VI. *Hearing thresholds (dB) corrected according to age.*

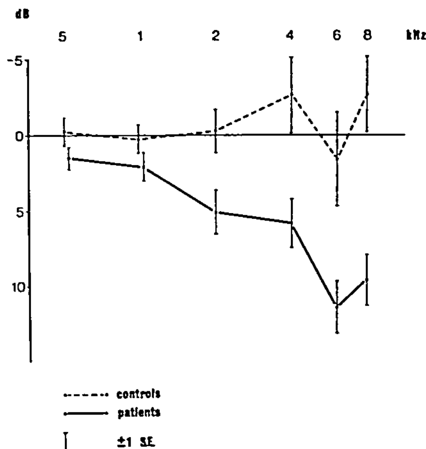


Fig 5 Age corrected mean audiograms

reflex test Stapedius reflex threshold was less than 65 dB SL (with a hearing threshold of 20 dB HL or more) in 38 of 98 ears (39 per cent) Thus, 42 ears (36 per cent) of 117 showed recruiting hearing losses, 7 ears under 50 years, 17 under 60 and 19 over 60 years Threshold tone decay was ≤ 30 dB in 109 ears (93 per cent), 35 – 40 dB in 5 ears and 70 dB in one ear

The hearing thresholds of 7 patients with aminoglycoside antibiotic history at 6 and 8 kHz (means 14 5 and 17 7 dB, ranges from 8 to 45 dB and from 8 to 54 dB respectively) were not significantly worse than those of rest of the group Testing patients without aminoglycoside history against reference subjects still gave significant differences at 6 and 8 kHz ($p < 0\ 0025$ and $p < 0\ 0005$ respectively)

Hearing losses at 6 and 8 kHz did not vary significantly between the furosemide group (13 4 and 9 4 dB) and the others (11 1 and 8 3 dB)

The relationship between the known duration of renal insufficiency and the degree of hearing loss was cleared up by comparing hearing at 6 and 8 kHz between patients with renal insufficiency of less than 5 years (13 5 and 10 3 dB) with that of those with a duration of more than 5 years (10 4 and 9 5 dB) No significant differences were noticed (Table VII)

The hearing thresholds of patients treated for arterial hypertension (11 9 and 9 4 dB) did not differ at 6 and 8 kHz from those of normotensive patients (10 1 and 8 1 dB)

No significant correlations were observed between serum creatinine or blood haemo

Duration	N	6 kHz Mean	S D	Min	Max	8 kHz Mean	S D	Min	Max
≤5	51	13 5	17 8	-25	57	10 3	18 1	-32	52
>5	66	10 4	19 6	-35	45	9 5	18 3	-32	54
		p>0 1				p>0 1			

Table VII *Known duration (years) of the renal insufficiency and age corrected hearing loss (dB) N = number of ears*

		N	6 kHz Mean	S D	Min	Max	8 kHz Mean	S D	Min	Max
Patients	Men	56	18 0	19 1	-21	57	12 7	17 6	-31	52
	Women	61	5 3	16 9	-35	47	6 7	18 2	-32	54
			p<0 001				p<0 1			
Controls	Men	14	2 3	23 6	-30	54	7 8	15 0	-32	24
	Women	26	1 2	16 8	-40	42	0 2	15 6	37	37
			p>0 1				p>0 1			

Table VIII *Sex and age corrected hearing loss (dB) N = number of ears*

	N	6 kHz Mean	S D	Min	Max	8 kHz Mean	S D	Min	Max
Pyelonephritis	62	7 6	18 1	35	57	6 1	18 3	-32	54
Others	55	14 8	18 7	21	47	14 1	17 1	31	47
		p<0 05				p<0 05			

Table IX *Chronic pyelonephritis and age corrected hearing loss (dB) N = number of ears*

globin level and hearing loss at the two highes frequencies (r ranged from 0.1 to 0.2).

In the patient group men had poorer hearing (18.0 and 12.7 dB) than women (5.3 and 6.7 dB) at 6 and 8 kHz ($p < 0.001$ and $p < 0.1$ respectively). No such differences between sexes could be verified in the reference series (Table VIII).

Age corrected hearing loss did not correlate any more clearly to age ($r = 0.2$).

Only the patients suffering from chronic pyelonephritis differed from the rest of the patients in regards to hearing at 6 and 8 kHz (Table IX), having better * hearing (7.6 and 6.1 dB) than others (14.8 and 14.1 dB). The mean age of this group was 47 years and that of others 53 years, sex distribution was 21 women/10 men.

No significant differences in mean hearing loss at 6 and 8 kHz could be traced between the various ENG groups (A: 10.2 and 9.3 dB, B: 10.8 and 10.0 dB, C: 14.8 and 11.6 dB) (Table X, Fig. 6). Dividing the patients in two groups according to mean hearing at 6 and 8 kHz (under and over 10 dB) gave an even distribution between the ENG groups (A: 11 and 8, B: 15 and 15, C: 7 and 7).

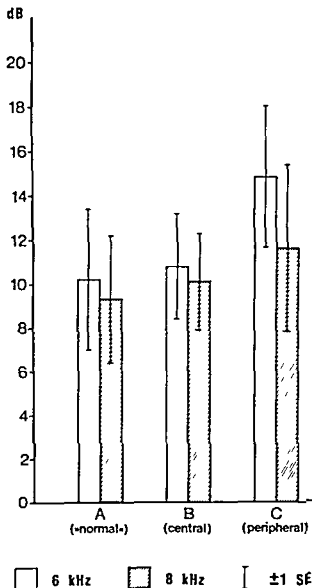


Fig. 6 ENG findings and age corrected hearing loss in patients.

		6 kHz				8 kHz			
	N	Mean	S.D.	Min	Max	Mean	S.D.	Min	Max
A ("normal")	38	10.2	20.0	-21	47	9.3	18.4	-31	54
B (central lesion)	59	10.8	18 8	-35	45	10.0	16.8	-32	44
C (peripheral lesion)	28	14.8	19.3	-25	57	11.6	20.0	-32	52

Table X. *ENG findings and age corrected hearing loss (dB). N = number of ears.*

VI DISCUSSION

The renal failure patients were mainly in better general condition in this than in previous investigations. The lowest accepted serum creatinine level was 200 mmol/l (2.3 mg/100 ml). Most recent studies deal with patients treated with haemodialysis (Beaney, 1964; Ransome et al., 1966; Yassin et al., 1966; Yassin et al., 1970*ab*, Mitschke et al., 1973, 1974 and 1975; Oda et al., 1976; Johnson & Mathog, 1976; Johnson et al., 1976, Thomsen et al., 1976; Henrich et al., 1977) Wigand et al (1972), however, selected patients on a basis similar to that used in this study using a somewhat higher serum creatinine threshold of 3 0 mg/100 ml. The less advanced stage of the renal disease permitted vestibular and audiological testing to be more thorough and accurate

In accordance to Wigand et al. (1972) questioning in present study did not reveal significantly more hearing problems in patients, 18 of 59, than in controls, 4 of 20 Complaints of balance disturbances were more frequent, 21 of 59, as noted by Yassin et al (1966) and by Wigand et al (1972)

Objective findings of vestibular disorders according to Wigand et al (1972) are rare, but Grahe (1924) found aberrations in caloric reactions in half of his "nephritis" patients Yassin's et al (1970*b*) results are not comparable with those of this study as 43 of their calorically tested 114 patients were in too weak a general condition to cope with pure tone audiometry

Nystagmus after head shaking must be considered pathological (Frenzel, 1955) It was observed in 17 of the renal patients and in none of the control subjects The difference was significant. The incidence of spontaneous and positional nystagmus was not significantly more frequent in the patient than in the control series. The proportion of disorders in vestibular system characterized by ENG abnormalities was significantly higher in the patient group. A central type of lesion of the vestibular system was noticed in half of the patients and in 25 per cent of the controls. A peripheral disorder was observed in 24 per cent of the patients but in none of the controls. The statistical significance was the same in central as well as in peripheral lesions.

The rotatory tests used revealed no significant differences between the two series. They were obviously less sensitive than the other ENG tests. The normal mean nystagmus threshold for angular acceleration lies between 0.2 and 0.8 °/s² using a modern electronically controlled turning equipment and nystagmography (Montandon et al., 1960 and 1969, Decher, 1962 and 1965; Bochenek & Gromowa, 1967; Hood, 1970, Virolainen, 1972) The mean thresholds of the present study (0.32 and 0.28 °/s²) thus fit in fairly well with the previous concepts. Slower accelerations of the chair than 0.2 °/s² would have been needed to establish the lowest individual thresholds.

As opposed to Beaney's (1964) concept the ototoxic antibiotics and diuretics could not be blamed for the majority of vestibular disorders

As one would assume, the frequency of central vestibular lesions grew with age. Higher serum creatinine concentrations were not connected with significantly more

vestibular disorders than lower concentrations The known duration of renal insufficiency did not correlate to the disorders of the vestibular system Moreover, no connection could be traced between ENG findings and blood haemoglobin level or treated arterial hypertension

The age corrected mean hearing thresholds of the control subjects (Fig 5) lie almost ideally near to the zero line, showing that the hearing of the control sample of this study represents well that of a greater sample (Palva A & Jokinen, 1970)

A high tone hearing loss was obvious in spite of the relatively small sample for audiological purposes (a greater sample was not available) This finding is in accordance with the results of previous investigations (Grahe 1924, Yassin et al, 1970*a*, Wigand et al, 1972, Kopsa et al, 1972, Mitschke et al, 1973 1974 and 1975, Johnson & Mathog 1976, Johnson et al, 1976, Henrich et al 1977)

The frequent recruitment phenomenon (36 per cent) the high speech discrimination scores and the low values of the threshold tone decay test give an indication of a primarily cochlear site of the lesion, which fits in well with previous concepts (Grahe, 1924, Yassin et al 1970*a*, Wigand et al, 1972, Kopsa et al, 1972, Mitschke et al, 1973) These results do not exclude the possibility of accentuated presbyacusis because the ordinary speech discrimination test is not effective enough in uncovering the poor discrimination ability of a person with presbyacusis (Jerger, 1973)

The aminoglycoside antibiotic and furosemide medication did not explain the hearing losses although that is suggested by many authors (Beaney, 1964, Bergstrom et al 1973, Quick 1976) Although according to present day practice advanced uraemia is treated with high doses of furosemide in milder cases as in this study the doses were most often so small that the temporary threshold shift demonstrated by ... Wigand et al (1972) with high intravenous doses did not appear

The present study confirmed the earlier observation that the serum creatinine level does not correlate with the hearing loss (Yassin et al, 1970*a*, Wigand et al, 1972, Johnson & Mathog 1976, Johnson et al, 1976) Hypertension and its treatment did not seem to play any role either The better hearing of the pyelonephritis patients is explained by the uneven sex distribution (21 women/10 men) The duration of the disease and age (after age correction) did not affect the hearing thresholds as noticed also by Johnson & Mathog (1976) In agreement with Grahe (1924) there was no connection between hearing loss and vestibular disturbances

In spite of the controversial results concerning the role of electrolyte balance in hearing loss (Yassin et al, 1970*a*, Wigand et al, 1972, Johnson & Mathog 1976, Johnson et al, 1976) electrolytes could not be included in this study The outpatients that were able to cope with the strain of the present examination were not supposed to suffer from any significant electrolyte disturbances The vestibular and hearing tests were carried out mainly in two separate sessions after contacting the patient during his visit at the renal unit This would have demanded at least two blood samples to avoid the error caused by possible changes in electrolyte concentrations That again would have diminished the number of volunteers dramatically Thus it was preferable to be satisfied with the laboratory results available

There are at least three types of cochleovestibular lesions which seem to be independent of each other and of the known variables of renal disease central disorder of the vestibular system which might be an indication of accentuated aging, peripheral vestibular disturbance and high tone loss

The results seem to exclude most of the imaginable single causes of the damages; hypertension along with the renin-angiotensin system, factors associated with haematopoiesis (haemoglobin level), accumulation of uraemic toxins (should be parallel to creatinine level and duration of the disease) and antimicrobic therapy (pyelonephritis group)

The role of hyponatremia and other electrolyte disorders in vestibular and cochlear disturbances needs futher investigation It would be of interest to clarify the possible connections with the early uraemic polyneuropathy observed in an advanced state of renal failure (Thomas et al , 1971)

VII CONCLUSIONS

1 Both central and peripheral vestibular disturbances occur frequently with renal insufficiency

2 *Even medically managed renal failure is* connected with high tone hearing loss of a primarily cochlear type

3 The development of vestibular disorders, even peripheral, and hearing loss seem to have different mechanisms

4 Ototoxic drugs alone cannot be blamed for these disturbances

5 The development of vestibular and hearing *disorders is obviously too complex to be* clarified with the methods used Interaction of known and/or unknown factors might explain the observations

The results seem to exclude most of the imaginable single causes of the damages: hypertension along with the renin-angiotensin system, factors associated with haematopoiesis (haemoglobin level), accumulation of uraemic toxins (should be parallel to creatinine level and duration of the disease) and antimicrobic therapy (pyelonephritis group).

The role of hyponatremia and other electrolyte disorders in vestibular and cochlear disturbances needs futher investigation. It would be of interest to clarify the possible connections with the early uraemic polyneuropathy observed in an advanced state of renal failure (Thomas et al., 1971).

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Experimentelle und klinische Untersuchungen zur Funktion des normalen, erkrankten und operierten Trommelfells

VON

JAN HELMS

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ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 350

Experimentelle und klinische Untersuchungen zur Funktion des normalen, erkrankten und operierten Trommelfells

VON

Jan Helms

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A. Vorbemerkung

„MIKROCHIRURGIE DES OHRES"

„Die Otochirurgie steht seit etwa 2 Jahrzehnten in dem bedeutendsten Umwandlungsprozeß ihrer Geschichte. Die Umwandlung betrifft in gleicher Weise die Prinzipien und die Technik der operativen Eingriffe. Nach dem Grundsatz, daß die Erhaltung des Lebens den Vorrang vor der Erhaltung einzelner Organe und ihrer Funktion haben müsse, richtete die ältere Ohrchirurgie ihr Augenmerk auf die Verhütung und Bekämpfung lebensgefährlicher Verwicklungen, d. h. der Meningitis, des Hirnabszesses und der Thrombophlebitis des Sinus. Die modernen Möglichkeiten einer antibiotischen Therapie haben die Gefahr lebensgefährdender Komplikationen zwar nicht beseitigt, aber doch weitgehend gebannt. Im Zuge einer natürlichen Entwicklung mußte dabei die Idee einer funktionellen Chirurgie in weit stärkerem Maße hervortreten." (Plester, 1970 (186))

Die wissenschaftlich und experimentell fundierten Anfänge der funktionellen Ohrchirurgie liegen in den 70er Jahren des vorigen Jahrhunderts. Kessel (129, 130) begann zu jener Zeit nach hörphysiologischen Überlegungen und Untersuchungen an Meerschweinchen mit der Stapeschirurgie bei Otosklerose am Menschen. Seinen Operationsprinzipien folgten Boucheron (31) und Miot (168). Zur Behandlung entzündlicher Mittelohrerkrankungen entwickelten Berthold (20), Kessel (131), Hoffmann (108) und v. Tröltsch (233), Ely (55) und Tangemann (219) Operationsverfahren, die den heutigen Techniken schon sehr nahe kamen.

Neben der Entwicklung chirurgischer Verfahren, die dem Funktionserhalt oder der Funktionsverbesserung des Ohres dienten, wurden wissenschaftliche Erkenntnisse zum Wirkungsprinzip des Gehörorganes erarbeitet.

Zur Klärung der Funktion des normalen und des veränderten Trommelfells soll die vorliegende Arbeit einen Beitrag leisten.

B. Einleitung

I DIE PHYLOGENESE DES MITTELOHRES ALS ANPASSUNGSVORGANG

Bei der Evolution der Lebewesen ergab sich mit der Entwicklung des Lebens außerhalb des Wassers unter anderem ein Problem in der Perzeption akustischer Reize aus der umgebenden Luft

Im Wasser fortgeleitete Schallreize können durch die z B in den Fischen entwickelten Sinnesorgane aufgenommen werden, da der Schall, ohne größere Widerstände überwinden zu müssen, in den im Wasser schwimmenden Organismus einzudringen vermag Die akustischen Widerstände zwischen Wasser und Organismus sind nur gering Die für die Fische entwickelten Sinnesorgane sind zur Aufnahme von Schallreizen aus der Luft dagegen nicht geeignet Der Übertritt von Schallenergie aus dem Medium Luft in das Medium Wasser oder in Analogie in das Säugetierinnenohr ist ohne Zwischenschaltung eines Transformations jedes mit einem Energieverlust von etwa ,9% verbunden (17, 123, 140 246 248 249)

Während der Entwicklung der Landlebewesen bildeten sich zwei unterschiedliche Prinzipien aus um den hohen Verlust von Energie bei der Perzeption von akustischen Reizen aus der Luft zu mindern Für die Insekten entstanden Sinnesorgane die ohne Zwischenschaltung von Flüssigkeit direkt der Luft zugänglich sind Für die Säugetiere blieb die Anordnung des Innenohres geschützt in der Tiefe des Schädels und umgeben von wässriger Flüssigkeit erhalten Es entwickelte sich ein Transformationsmechanismus der auf der einen Seite durch eine Angleichung eines eigenen Schallwellenwiderstandes an die Werte für Luft zur vollständigen Übernahme der Schallenergie aus der Luft geeignet war der auf der anderen Seite durch eine Druckerhöhung aber erreichte, daß die auftreffende Energie ohne wesentliche Reflektion an das Innenohr weitergegeben werden konnte Dieser *Transformationsmechanismus* ist das Mittelohr des Säugetiers (140)

II ENTWICKLUNGSGESCHICHTLICHE DATEN ZUR MITTELOHRENTSTEHUNG

Reptilien und Vögel besitzen ein einfacher gestaltetes Ohr als Säugetiere Im komplexen Transformationsmechanismus zwischen Luft und Perilymphe des Innenohres weist es anstelle der Gehörknöchelchenkette des Saugerohres ein säulenförmiges, nur leicht geknicktes Knöchelchensystem, die Columella auf Für innerhalb der Tierart wichtige Schallreize konnte jedoch eine sehr wirksame Transformationsleistung nachgewiesen werden (123, 202, 248)

Während bei Fischen das Mittelohr noch vollständig fehlt und aus den erwähnten Gründen nicht erforderlich ist, bilden sich aus Anteilen der ersten beiden Kiemenbögen in der weiteren aufsteigenden Tierreihe die einzelnen Komponenten des Mittelohrtransformationssystems aus Die Paukenhöhle entwickelt sich aus der ersten Kiemenspalte Die Entwicklung der einzelnen Bausteine des Mittelohres in den verschiedenen Wirbeltierklassen schildert detailiert H M de Burlet (36) Witschi (Z f Naturforschung 4 b 4 (1949)) analysierte eingehend die Entwicklungsvorgänge am Froschohr einem besonders interessanten Objekt da es sich während der Metamorphose des Tieres vom Wasser auf das Landleben umstellen muß Die Untersuchungen von Filogamo (65)

die sich insbesondere mit der Entstehung des Trommelfells befassen, zeigen, daß bei Amphibien diese Membran noch ausschließlich von radiären Fasern aufgebaut ist und eine annähernd ebene Fläche darstellt, während sich durch die Ausbildung zusätzlicher, insbesondere circularer Fasersysteme bei den Wirbeltieren eine Trichterform des Trommelfells ausbildet. Diese Pseudokonusform des Trommelfells ist, wie Secondi (208) zeigen konnte, bei allen Wirbeltieren ausgebildet und wird durch radiär-, circulär- und parabelförmig verlaufende Faseranordnungen aufrecht erhalten. Der Eintritt von Luft aus dem Epipharynx über die Eustachische Tube in den Mittelohrraum unter Verdrangung des hier bei der Ausbildung der Kiementasche noch bestehenden embryonalen Füllgewebes wurde zuletzt ausführlich von Proctor 1964 (195) studiert.

Nach Altmann (5) bilden sich die Gehörknöchelchen teilweise aus dem 1. und teilweise aus dem 2. Kiemenbogen aus. Die oberen Anteile der Ossicula, also Hammerkopf und Amboßkörper, entwickeln sich aus Zellhaufen des Mesenchyms im ersten Kiemenbogen, während Hammergriff und langer Amboßfortsatz aus Zellansammlungen im Mesenchym des zweiten Kiemenbogens hervorgehen (s. a. 112). Mit dem langen Amboßfortsatz, verbunden durch das Amboß-Steigbugelgelenk, entwickelt sich die Stapesanlage zwischen der 6. und 8. fetalen Lebenswoche.

Die für den Otologen und Otochirurgen wichtigen Befunde der Embryologie des menschlichen Mittelohres stellten Kobrak (140) und Altmann (5) ausführlich zusammen.

Bei der Geburt hat das Trommelfell seine spatere Große fast vollstandig erreicht. Lediglich die Pauke ist pneumatisiert. Die Ausbildung der lufthaltigen Zellen im Warzenfortsatz schließt mit der Pubertat ab.

III. ANGABEN ZUR ANATOMIE DES MENSCHLICHEN MITTELOHRES

Die Kenntnis der Anatomie des Mittelohres geht auf Hippokrates (zit. n. 44) zuruck, der das Trommelfell entdeckte. Vesalius (237) beschrieb 1543 Hammer und Amboß. 1546 fand Ingrassia den Stapes. Schon 1573 findet sich bei Coiter (43), nach der vorangehenden Entdeckung der Tuba auditiva durch Eustachius 1564, eine ausführliche Beschreibung der Mittelohranatomie. Das Trommelfell wird durch Luftdruckschwankungen bewegt (19, 43, 44, 45, 46, 153).

Helmholtz (100) erarbeitete um die Mitte des vorigen Jahrhunderts grundlegende Erkenntnisse uber die Physiologie des Mittelohres. Er wurde aus diesem Grunde auch zu subtilen anatomischen Untersuchungen angeregt und publizierte sie zusammen mit seinen physiologischen Daten als ersten Artikel des neu gegrundeten Archivs für die gesamte Physiologie des Menschen und der Tiere Bd. 1, Seite 1, 1868 (100). Insbesondere kam es Helmholtz darauf an, neben den Konturen von Trommelfell und Gehörknöchelchen die Art der Befestigung an den Wandungen des Mittelohres zu beschreiben, um daraus Informationen über die Funktion abzuleiten. Der Bandapparat, der die Gehörknöchelchenkette hält, und die Funktion der Gelenke zwischen den Ossicula wurden von ihm untersucht. Er bezeichnete den straffen Bandapparat in der vermuteten Drehachse der Gehörknöchelchenkette als „Achsenband".

Das Trommelfell schließt das Mittelohr nach lateral ab. Es ist konusförmig einwärtsgewölbt, grau schimmernd und etwa kreisrund. In einem nach oben vorne weisenden Radius ist es mit dem Hammergriff verwachsen, wobei nur der laterale Aspekt des Hammergriffes Kontakt mit dem Trommelfell bekommt. Das Trommelfell selbst verschließt den außeren Gehörgang schragstehend. Der vorne unten liegende Teil steht medialer als das hintere obere Areal. Der Durchmesser des Trommelfells betragt von oben nach unten 9–10 mm und von hinten nach vorne etwa 7–9 mm. Diese Angaben, die Helmholtz (100) in Übereinstimmung mit v. Trolsch (233) publizierte, wurden bis heute immer wieder bestätigt. Neuere Messungen an Japanern (137)

weisen etwas kleinere Werte auf. Die Dicke des Trommelfells beträgt etwa 0,7–1 mm (100, 137).

Der craniale Anteil der Trommelfellfläche in der Incisura Rivini ist die Shrapnell'sche Membran. Dieser kleinere Teil des Trommelfells weist im Gegensatz zum Hauptanteil keine straffe Bindegewebsschicht auf, sondern besteht im wesentlichen aus äußerer Haut und der die Pauke auskleidenden Schleimhaut (140).

Helmholtz (100) beschreibt die akustisch wirksame Pars tensa des Trommelfells folgendermaßen: „Die mittlere festere Schicht des Trommelfells ist eine fibröse Haut, die teils aus radiär, teils aus circular verlaufenden Fasern besteht. Die radiären Fasern liegen auf der äußeren Seite, die circularen auf der inneren Seite dieser Schicht. Für jene bildet in der vorderen Hälfte das Ende des Hammerstiels den Mittelpunkt ihrer Ausstrahlung. Auf der hinteren Seite dagegen laufen sie mehr parallel von der ganzen Fläche des Hammerstieles aus. Ihre Schicht ist längs des Randes am dünnsten und verdickt sich allmählich gegen den Hammerstiel hin, wo sie sich mehr zusammendrängen.

Die circularen Fasern bilden im Zentrum des Trommelfells eine sehr dünne Schicht, die sich gegen die Peripherie hin allmählich verdickt, die äußere Peripherie aber frei läßt oder wenigstens wieder viel dünner wird als sie in der Mitte ist. Am Rivini'schen Ausschnitt sind die Ringfasern ziemlich stark entwickelt, atlasglänzend, und bilden hier den Befestigungsstrang, der den festeren Teil des Trommelfells nach obenhin begrenzt; sie schneiden sich hier unter einem ziemlich kleinen, spitzen Winkel mit den radiären Fasern, die an dieser Stelle nicht vom Nabel, sondern vom kurzen Fortsatz des Hammer ausstrahlen. Hier mischen sich dann auch die unregelmäßig durcheinandergeschlungenen Cutisfasern ein.

Die Sehnenfasern dieser Schichten sind sehr feste, straffe Bänder, dicht nebeneinanderliegend und jeder Dehnung einen sehr großen Widerstand entgegensetzend. Sie unterscheiden sich durch ihren sehr großen elastischen Widerstand wesentlich von den viel nachgiebigeren gelben elastischen Geweben. Die Substanz des Trommelfells schwillt in Essigsäure und Kalilösungen, wie es das Sehnengewebe, nicht aber das elastische Gewebe thut. Ich fand, daß sie wie Sehnengewebe durch Kochen in verdünnter Kalilösung schnell vollständig aufgelöst wird, wobei nur geringe Reste elastischen Gewebes zurückbleiben, welches teils deutlich noch Gefäßröhren erkennen läßt, teils auch eine sehr dünne continuirliche Membran, die wahrscheinlich die Grundlage des Schleimhautblatts an der inneren Seite des Trommelfells bildet.

Diese Art der Zusammensetzung des Trommelfells ist für seine mechanische Leistung von größter Wichtigkeit, wie die Folge zeigen wird. Es ist nicht als elastisch nachgiebige, sondern als fast unausdehnbare Membran aufzufassen. Seine sehr geringe Nachgiebigkeit zeigt sich auch, wenn man es entweder in seiner natürlichen Befestigung oder nachdem man es gelöst und auf eine Glasplatte ausgebreitet hat, mit Stecknadeln zerrt. Es zieht sich nicht aus wie ein Kautschukblatt oder wie ein Stück aufgeweichter tierischer Blase, sondern es widersteht dem Zuge sehr kräftig und bildet Falten rings um die gezerrte Stelle wie eine Collodiummembran."

Diese 1868 gewonnene anatomische Kenntnis des Trommelfellaufbaus muß auch heute noch als umfassend angesehen werden. Nachuntersucher haben die Richtigkeit der Anschauungen wiederholt bestätigt (86, 132, 137, 190, 191, 235).

Eine noch weitere Aufschlüsselung der einzelnen Faserbündel, die sich in der Lamina propria der Pars tensa des Trommelfells unterscheiden lassen, wurden Mitte diesen Jahrhunderts von Fumagalli (73), Secondi (208) und mit elektronenoptischen Untersuchungsmethoden von Johnson (119), Lim (147, 149), Kawabata (125) und Shimada (211) angegeben.

Johnson (119) fand zusätzlich, daß die Bindegewebsfasern nach hochspannungspapierelektrophoretischer Auftrennung der

Einzelkomponenten eine besondere Kollagenart darstellen müssen Es wird vermutet, daß die Trommelfellfasern für ihre Funktion besonders spezialisiert sind Die Gefäßversorgung (90, 238) zeigt ein arterielles, radiär angeordnetes Netzwerk zwischen äußerer Bedekkung und Tunica propria Das venose Blut fließt über Mucosagefäße der Pauke ab Die nervöse Versorgung (37, 49, 124, 160) gewährleistet außen ein Nervengeflecht aus dem Nervus auriculo temporalis und innen ein ähnliches Geflecht aus den Jakobson'schen Nerven des Glossopharyngicus in der Pauke Zusätzlich besteht noch ein markloses Nervengeflecht in der Tunica propria (49)

Das Trommelfell schließt das Mittelohr weitgehend gasdicht ab (51) Die Diffusionsmoglichkeit für N_2 O_2 CO_2 ist 1 2·70, wobei insgesamt nur etwa 0 5–1 % der uber die Tube ventilierten Luftmenge von 1–2 ml täglich durch das Trommelfell abdiffundieren kann

Die äußere Form des Trommelfells mit der Ausbildung eines einwartsgerichteten Trichters, dessen Flachen zur Trichterachse hin vorgewölbt sind, wurde besonders eingehend von Kirikae (137) beschrieben In seiner ausführlichen Monographie finden sich die modernen anatomischen Kenntnisse uber den Aufbau des Trommelfells umfassend zusammengestellt Weitere Einzelheiten der Mittelohranatomie wurden zusammengetragen von Beck (9, 10) u a (33, 43, 46, 100, 132, 137, 140, 148, 191, 206 213, 235)

IV FUNKTION DES MITTELOHRES ALS TRANSFORMATIONSGLIED ZWISCHEN SCHALLREIZ UND INNENOHR

Die unterschiedlichen Impedanzen von Luft und eines flussigen Mediums, wie es das Innenohr zeigt, machen einen Transformationsmechanismus notwendig, um einen Ubertritt von moglichst viel Reizenergie von dem Medium Luft in die Cochlea zu gewahrleisten Dieses Transformationsglied stellt das Mittelohr dar (123)

Das äußere Ohr trägt bei manchen Tieren zur Optimierung der Schallperzeption bei Nur Saugetiere besitzen Ohrmuscheln Diese sind beweglich bei Tieren, die im Hochfrequenzbereich hören Unbeweglich sind die Ohrmuscheln bei weniger gutem Hörvermogen für hohe Frequenzen, z B beim Menschen (123) Im außeren Gehörgang kommt es oberhalb von 1000 Hz zu einer Schalldruckzunahme (17, 26) Eine Verengung des Gehörganges kann zur Verminderung des Schalldruckes am Trommelfell in Abhängigkeit von der Frequenz führen (253)

Nach Wever und Lawrence (246, 248) wird ein Schallreiz vom Medium Luft in ein anderes Medium entsprechend dem Verhältnis der akustischen Widerstande zueinander aufgenommen Der akustische Widerstand eines Mediums hängt ab von der Dichte und der Elastizität

Für den verwandten mechanischen Widerstand gelten folgende Beziehungen (246, 248)

R = Impedanz in mech Ohm (g cm^{-2} sec^{-1})
p = Dichte
S = Elastizitat
r = Impedanzrelation
T = Im zweiten Medium aufgenommene Energie

$$R_1^{(Luft)} = \sqrt{p_1 S_1}$$

$$R_2^{(Wasser)} = \sqrt{p_2 S_2}$$

$$r = \frac{R_1}{R_2}$$

$$T = \frac{4r}{(r+1)^2}$$

Auftreffende Energie = 1
Reflektierte Energie = $1-T$

Aus diesen Gleichungen folgt

1 Sind die akustischen Widerstände gleich, also $R_1=R_2$, so wird auch $r=1$ und damit

$T = 1$ Dies bedeutet, daß die gesamte auftreffende Energie aufgenommen wird Eine Reflektion findet nicht statt

2 Ist R_2, also z B die Impedanz des Innenohres, großer als R_1, also als der Wert fur die Luft, so wird T kleiner als 1 Ein entsprechend großer Anteil der auftreffenden Energie wird reflektiert

Werden die Werte für Luft und Wasser in die genannten Gleichungen eingesetzt, so resultiert folgendes

$$r = \frac{\text{Impedanz Wasser}}{\text{Impedanz Luft}} = \frac{148\,000}{41{,}5} = \sim 3\,600$$

$$T = \frac{4 \times 3\,600}{(3\,600 + 1)^2} = \sim 0{,}001$$

Der Betrag der reflektierten Energie ware bei direktem Auftreffen von Luftschall auf das Innenohr $1 - 0{,}001 = 0{,}999$ Etwa 99,9% der auftreffenden Energie wurden also reflektiert Die physikalischen Parameter des Mittelohres verhindern dies Das Trommelfell setzt den Schallwellen praktisch keinen Widerstand entgegen Seine Impedanz ist etwa so groß wie die der Luft Es nimmt die Schallenergie vollstandig auf

Die Flachenrelation Trommelfell/Stapesfußplatte betragt fur den Menschen etwa 17 1 und für die Katze (123) 25 1 Die wirksame Trommelfellflache nimmt nach v Bekesy (17) etwa 70% der Gesamtflache ein Durch weitere sich mit diesem Haupttransformationsfaktor multiplizierende Untersetzungsprinzipien, die innerhalb des Mittelohres verwirklicht sind, wird z B bei Katzen eine nahezu ideale Uberbruckung des großen Impedanzunterschiedes zwischen Luft und Innenohr erreicht

Die Impedanzrelation Innenohr/Luft betragt ca 2 500 Wever (249) konnte zeigen daß ein Transformationsglied mit dem Faktor $\sqrt{2\,500} = 50$ das Optimum darstellen wurde Dieser Transformationsfaktor ist z B im Katzenohr verwirklicht Multipliziert man die Trommelfell/Stapesrelation von 25 mit dem Untersetzungsverhältnis von 2 innerhalb der Gehorknochelchenkette, so ergibt sich dieser Faktor 50 fur die Katze (249)

Das Prinzip dieses Wirkungsmechanismus hatte schon Helmholtz 1868 (100) erkannt, wie moderne Untersuchungen beweisen (11, 13, 17, 23, 24, 114, 123, 136, 137, 163, 225, 227, 228, 229, 230, 246) Helmholtz hatte damit schon fruher geaußerte Ansichten (43, 46, 47, 232) bestatigt Erkenntnisse, daß die Gehorknochelchenkette nicht zur Ubertragung des Schalls geeignet sei, wie sie vereinzelt um die Jahrhundertwende geaußert wurden (21, 22, 245), sind endgültig als unbegrundet anzusehen, wenn auch in Sonderfallen eine Schallubertragung nicht uber die Gehorknochelchenkette, sondern uber die Luft im Mittelohr direkt auf das runde Fenster moglich ist (Sonoinversion)

Die Schwingungsamplituden bei Schalldrucken in der Nahe der Horschwelle liegen im molekularen Bereich, wie v Békésy (17) zeigen konnte Erst durch komplizierte mathematische Gleichungen wurde die Funktion des Drucktransformators Mittelohr darstellbar (11, 13, 79, 87, 122, 123, 163, 172, 173, 178, 249)

Fur die Klinik ergeben sich Probleme bei Defekten der Einzelteile des Mittelohrschalldrucktransformators Unter pathologischen Bedingungen kann die gleichzeitige Beschalfung beider Innenohrfenster zu einer Schwerhorigkeit führen Die Minderung des Schalldruckes an einem der Fenster durch einen Schallschutz (Moritz, Wullstein (253)) kann das Horvermogen wieder bessern Unterschiedliche Grade der Schwerhorigkeit resultieren aus verschiedenen Defekten innerhalb der Gehorknochelchenkette und des Trommelfells sowie bei Storungen im Bereich der Fensternischen Aufgabe der modernen Mikrochirurgie des Ohres ist, diese pathologischen Verhaltnisse zu bessern

1. Transformationscharakteristik

Der Mathematiker B Riemann (zit n 100) machte sich als erster Gedanken uber den

Reiztransport im Mittelohr Er forderte, daß die Druckänderung der Luft in jedem Augenblicke in konstantem Verhältnis vergrößert auf das Labyrinthwasser ubertragen werden

Helmholtz (100) formulierte ähnlich, daß die Genauigkeit der Wahrnehmung es erfordere, daß jeder Ton von konstanter Hohe immer wieder, sooft er vorkomme, eine Empfindung von gleicher Art und Intensität auslösen müsse

Bei Untersuchungen über die Beziehung zwischen Schalldruck am Trommelfell und Stapesbewegungen in Abhängigkeit von der Frequenz und vom Schalldruck zeigte v Békesy (14), daß Reize bis uber die Schmerzgrenze hinaus, also in dem Bereich von 140 dB, noch als physiologisch angesehen werden mussen Eine lineare Ubertragung bezogen auf die Frequenz, ließ sich oberhalb von etwa 500 Hz im Tierversuch beobachten (122, 123) Für den Niederfrequenzbereich um 300 Hz wurde der Nachweis einer linearen Ubertragung bis zu Drucken von 140 dB (88) bzw 104 dB (199) erbracht Oberhalb dieser Lautstärken kommt es insbesondere im Amboß-Stapesgelenk zu Dislokationen, die eine weitere lineare Ubertragung verhindern

2. Trommelfellfunktion

„Die wichtigste Function des Trommelfells besteht in der Ubertragung seiner durch die Schallwellen erregten Schwingungen auf die ubrigen schallleitenden Theile des Gehororganes, außerdem dient das Trommelfell als Schutzorgan für die Paukenhöhle" (Urbantschitsch, 1880 (235)) Diese Lehrbuchdarstellung gilt noch heute (24, 140, 167, 169, 171, 190, 226)

Bei ubergroßen Schalldrucken kommt es zur Perforation des Trommelfells, das nicht nur primar eine Schutzfunktion gegenüber dem Mittelohr, sondern in diesen Situationen der Schalluberlastung auch eine Schutzfunktion gegenuber dem Innenohr als Sollbruchstelle einnimmt (11, 12)

Ubereinstimmend geben v Bekesy (17) und Wever und Lawrence (246) die wirksame Trommelfellflache mit etwa 75% der Gesamtflache an Die Wirkung wird als stempelartig angesehen, die Trichterform sei lediglich zur Versteifung des Flachenzentrums ausgebildet, ahnlich wie auch moderne Lautsprechermembranen

Direkte Messungen zur Schwingungsamplitude des Trommelfells führte als erster v. Békésy (13) durch Modifiziert werden die Schwingungsmöglichkeiten des Trommelfells durch den Zug des Musculus tensor tympani, der den Hammergriff nach medial verlagert Das Trommelfell wird dadurch gespannt und besser geeignet, höhere Frequenzen zu ubertragen (86, 133, 232)

Im Gegensatz zu den Untersuchungen von v Békesy (17) konnte Kinkae (137) zeigen, daß die Schwingungen des Trommelfells nicht gleichmäßig über die Membran verteilt sind In Abhängigkeit von der Position des Hammergriffes schwingt das Areal, das eine kleinere Entfernung zwischen dem Hammergriff und dem Trommelfellrand aufweist, weniger als die dann gegenüberliegende größere freie Trommelfellflache Die Interpretation dieser Befunde blieb unklar

Eine Störung der Trommelfellfunktion wird naturgemäß besonders von Perforationen verursacht Lokalisation und Größe der Perforation verandern die Reizweiterleitung auf die Gehörknöchelchen Eine Perforation im Shrapnellbereich zeigt eine geringe Beeinträchtigung der normalen Übertragungseigenschaften, weil dieser Bereich auch normalerweise zum Reiztransport nicht beiträgt

Cancura (38) versuchte, den Effekt der Lokalisation einer Perforation in der Pars tensa näher zu analysieren Er fand, daß Defekte in der vorderen Trommelfellhalfte besonders den Frequenzbereich über 2000 Hz beeinträchtigen Payne und Githler (181) fanden ahnliches an Katzen

An ausführlichen Modellversuchen konnten McArdle und Tonndorf (164) zeigen, daß Perforationen in der Pars tensa des Trommelfells bei sonst normalen Verhältnissen die Lei-

stungsfähigkeit des Mittelohres besonders bei Zunahme der Perforationsgroße von 0 auf 10% der Trommelfellflache storten Eine Ab hangigkeit von der Lokalisation der Perfora tion zeigte sich nicht Daruber hinausgehende Großenzunahmen der Perforation bewirkten dann keine wesentliche Horverschlechterung mehr Wahrscheinlich kommt die Storung der Mittelohrfunktion durch eine Schalldruckzu nahme im Paukenbereich durch die Perfora tion hindurch zustande (164)

Rekonstruierte Trommelfelle zeigen in Abhangigkeit von den Vernarbungen unter schiedliche Grade der Angleichung an die Normalsituation (229 255) Die Bedeutung der Schallprotektion für das runde Fenster wurde ausführlich auch von Schmitt (205) untersucht Seine Darstellungen bestatigen aus theore tischer Sicht die klinische Erfahrung daß durch eine gute Schallprotektion uber dem runden Fenster eine hochgradige Schwer horigkeit auf mittelgradige Werte reduziert werden kann

Den Verschluß einer Trommelfellperfora tion mit Fremdmaterial zur Horverbesserung empfahlen schon Banzer (1640 (6)) Yearsley (1848) und Toynbee (1853 (138)) Alexander (4) verwendete eine 0 01 mm starke Silber folie

Kontroverse v Bekesy – onndorf/Helmholtz

1868 beschrieb Helmholtz die Bewegungsform des Trommelfells als einen komplizierten Vor gang Nach seinen Untersuchungen (100) soll te die auswartskonvex gebogene Form der Radiarfasern die am Hammergriff ansetzen ein besonderes Hebesystem darstellen das den Schalldruck vom Trommelfell zum Ham mergriff hin verstarkt Dieses trommelfell eigene Hebelsystem in dem das Prinzip ge bogener Flachen verwirklicht sei sollte wesentlich zum Transformationsmechanismus des Mittelohres beitragen Eine direkte Messung der Bewegungsform des Trommel fells war Helmholtz noch nicht moglich Er schloß aus Volumenanderungen im Gehorgang bei Druckanderungen innerhalb des Innen ohres und intaktem Mittelohr daß dieses trommelfelleigene Hebelsystem existieren musse (100) Die notwendigen mathema tischen Formulierungen die dieser Theorie zugrunde liegen sind von Marquet und Mit arbeitern 1973 zusammengestellt worden (163) Guelke und Keen (86) fanden 1949 die Helmholtz sche Theorie bestatigt Auch in der Monographie von Kirikae (1960 (137)) finden sich Hinweise für die Richtigkeit der Helm holtz schen Theorie Durch umfangreiche Un tersuchungen mit der modernen Technik der Laser Holographie konnten Tonndorf und Khanna seit 1970 (136 227 228) eine Schwin gungsform im Trommelfell beobachten die ebenfalls die Ansichten von Helmholtz stutzt

Im Gegensatz zu diesem Prinzip der geboge nen Flachen als Trommelfelldruckverstar kungsmechanismus und im Gegensatz damit zu einem trommelfelleigenen Hebelsystem fand v Bekesy (13 17) eine andersartige Be wegungsform des Trommelfells Er war der erste der die Auslenkungen dieser Membran direkt mittels kapazitativer Sonden messen konnte Seine Befunde fuhrten zu dem Schluß daß das Trommelfell wie eine Klappe um die gemeinsame Achse der Gehorknochelchen dem Achsenband schwinge wobei die großte Auslenkung am Unterrand des Trommelfells gegenuber dem Umbo zu beobachten sei Diese Faltenbildung und die Ansicht einer klappenartigen Bewegung des Trommelfells wurde international akzeptiert Kobrak (140) konnte die Faltenbildung beobachten Groen (85) bestatigte ihre Existenz Guelke und Keen (86) konnten bei der Messung von Bewegungs vorgangen in der caudalen Trommelfellhalfte ebenfalls in dem von v Bekesey beschriebe nen Bereich das Maximum der Auslenkung feststellen Die für diese Schwingungsform notwendigen mathematischen Formulierun gen finden sich ebenfalls in der Monographie von Marquet und Mitarbeitern (163)

Johnstone (123) kommt in einer großen Ubersicht uber die Physiologie des Mittel ohres unter Einschluß eigener Experimente

mit sehr großer Empfindlichkeit (Mossbauereffekt) zu dem Ergebnis, daß die Diskrepanz zwischen den Messungen von v. Bekésy (17) und auch Møller (169) auf der einen Seite und Tonndorf und Mitarbeiter auf der anderen Seite bisher nicht entschieden werden konnte. Rein mathematische Ableitungen (58) weisen den Nachteil auf, daß die anatomischen Voraussetzungen nicht exakt berucksichtigt werden (123).

b. *Transformationsfaktor des Trommelfells*

Aus den Untersuchungen von v. Békesy folgt, daß ein Transformationsfaktor zwischen Trommelfell und Hammergriff nicht besteht. Lediglich die Flachenrelation wirksame Trommelfellflache/Steigbugelfußplatte geht in den Drucktransformationsmechanismus des Mittelohres ein.

Nach den Untersuchungen von Helmholtz ist ein Drucktransformationsfaktor 2 im trommelfelleigenen Hebelsystem anzunehmen. Zu ahnlichen Großenangaben des Faktors kommen Tonndorf und Khanna nach Messungen mittels der Laser-Holographie (227, 228, 229). Aus den Ableitungen von Mach und Kessel (157) folgt, daß dieser trommelfelleigene Hebel abhangig sein musse von dem Krummungsgrad der Trommelfelloberflache. Marquet und Mitarbeiter (163) änderten in Experimenten diesen Krummungsgrad. Sie konnten im Modell zwar keinen Wechsel des auf den „Hammergriff" wirkenden Druckes feststellen, verzichteten aber auf physiologische Untersuchungen. Hartman (94) wies in den Berechnungen von Helmholtz einen Rechenfehler nach. Das Prinzip der Helmholtz'schen Annahmen blieb erhalten.

3. Funktion der Gehorknochelchenkette

Die Funktion der Gehorknochelchenkette wurde in der Mitte des 16. Jahrhunderts erstmals von Colombo (45) als Ubertragungsmechanismus der Trommelfellschwingungen auf das Hororgan erkannt und beschrieben. Helmholtz (100) und Mach und Kessel (158) gewannen nach unzureichenden Beobachtungen in den dazwischenliegenden Jahrhunderten (47) durch intensives Studium von Felsenbeinpräparaten und Experimenten auch an Modellen die noch heute gultigen Kenntnisse über die Mechanik der Gehörknöchelchen (101). Aber noch 1913 wurden Vorstellungen über den Schalltransport durch die Gehörknochelchenkette zum Innenohrs z. B. von Bayer (22) angegriffen. Er nahm eine Ubertragung durch die Luft der Pauke und das ovale Fenster an.

Die besondere Bedeutung der Gehorknochelchen liegt, wie moderne Untersuchungen von Wever und Lawrence (246, 247), Schmitt (205), Kobrak (140), v. Bekesy (17) und vielen anderen immer wieder gezeigt haben in der Schallubertragung vom Trommelfell auf die Stapesfußplatte, womit die Gehorknochelchenkette ein wichtiges Glied im Mittelohrdrucktransformationsmechanismus darstellten. Fehlt die Ankoppelung des Trommelfells an das Innenohr, so resultiert eine hochgradige Schalleitungsschwerhörigkeit. Die in der Gehörknöchelchenkette vorhandenen Gelenke führen zwar bei der Ubertragung von Schallreizen zu einer gewissen Verzerrung, doch liegen die daraus resultierenden Störgerausche in ihrem Pegel so niedrig, daß sie in der Regel vernachlassigt werden können (11). Die Anordnung der Massen der Gehörknochelchen um ihre Schwingungsachsen bewirkt, daß bei schnellen Kopfbewegungen keine Schwingungen der Ossicula entstehen und daß auch bei feinen Bewegungen des Kopfes, wie z. B. bei der Entstehung von Kau- oder Atemgeräuschen, keine Bewegung der Gehorknöchelchen resultiert (85). Ähnliche Verhältnisse liegen nicht nur bei Saugetieren, sondern auch bei Vogeln und Amphibien vor (1, 41, 123, 202). Eine Zusammenstellung der bis heute akzeptierten oder begründet diskutierten Ansichten findet sich bei Marquet und Mitarbeitern (1963), deren Arbeit sich insbesondere mit der Änderung der Ubertragungseigenschaften des Mittelohres unter Anspannung der Mittelohrmuskeln beschaftigt. Die

Kontraktion dieser Muskeln, des M tensor tympani, der den Hammerhals nach medial verlagert, und des M stapedius, der den Stapeskopf nach hinten unten zieht, bewirkt eine wesentliche Spannungsveranderung innerhalb der Gelenke und des Trommelfells (85, 172) Die Beobachtungen von Tonndorf und Khanna (231) haben erkennen lassen, daß innerhalb der Gehorknochelchenkette in Abhangigkeit von der Frequenz und als Variation zwischen unterschiedlichen Beobachtungsobjekten Unterschiede der Bewegungsform vorkommen konnen

Die Gehorknochelchenkette bewegt sich bei Beschallung nicht immer als winkelförmiger Hebel um die Rotationsachse des Achsenbandes

a *Hebelwirkung*

Die Form der Gehörknochelchenkette mit den beiden Hebelarmen Hammergriff und langer Amboßfortsatz haben schon früh die Vermutung nahegelegt, daß außer der reinen Fortleitung des Schallreizes hier eine Drucktransformation stattfinden könne Diese Hebelubersetzung wurde 1929 von Dahmann (48) mit einem Wirkungsgrad von 1 1,3 bestimmt Kobrak (140) konnte zeigen, daß mit zu [illegible] Belastung des Hammers die Mit[illegible]ung des Amboß abnimmt Nach Stuhlmann (218) differiert das Hebelverhaltnis zwischen Hammer und Amboß bei Ein und Auswartsbewegung Bei Einwartsbewegung betragt es 1 1, bei Auswartsbewegung 2 1 Helmholtz (100) bestimmte ahnlich wie Dahmann eine Hebelubersetzung von 1 1,5

Für die Katze konnte Tonndorf (225) ein Hebelverhaltnis Hammergriff/Steigbügel von 2,2 1 finden Das Hammer Amboß Gelenk erwies sich dabei fest fur hohe Frequenzen und verschieblich für tiefere Tone Diese Tatsache führte dazu, einen Drucksteigerungsfaktor über die Gehorknöchelchenkette als zwar möglich aber nicht notwendig und nicht immer vorhanden anzusehen Die Wiederdeckung des trommelfelleigenen Hebelsystems im Prinzip der gekrummten Membranen von Helmholtz machte eine Druckverstarkung innerhalb der Gehorknochelchenkette überflussig, da eine ausreichende Vergroßerung der Flächenrelation Trommelfell/Stapesfußplatte über das zusatzliche trommelfelleigene Hebelsystem gewahrleistet wurde

b *Schutzwirkung*

Die Notwendigkeit von Schutzmechanismen im Mittelohr, die eine Ubertragung von uber großen Schallreizen auf das Innenohr vermeiden, wurden erstmals von Betzold 1908 diskutiert (24) Neben der Schutzfunktion des Trommelfells selber, das als Sollbruchstelle einreißen kann, ist insbesondere der Schlupf in den Kettengelenken als ein solcher Schutzfaktor anzusehen (24, 100, 246) Auch bei Amphibien und Vögeln besteht zwischen Columella und Extracolumella eine Struktur analog dem Amboß Stapes Gelenk, die als Sollbruchstelle angesehen werden kann (250)

Nach v Bekesy zeigt der Stapes im ovalen Fenster eine stempelartige Bewegung, die bei großen Lautstarken in eine Taumelbewegung umschlagt, so daß das Hubvolumen der Perilymphe nicht proportional zu den großen Schalldrucken am Trommelfell steigt Auch dieser Mechanismus kann als eine Art Schutzfunktion angesehen werden

4. Bedeutung der Tubenfunktion fur die Mittelohrleistung

Eustachius beschrieb 1564 die nach ihm benannte Tuba auditiva Schellhammer (1649–1716) erkannte, daß die Tube zur Ventilation des Mittelohres diente, und Valsalva (1666–1723) wies nach, daß die Tubenfunktion für das Horen wichtig sei (25, 253) Eine besondere Bedeutung erlangte die Feindiagnostik der Tubenfunktion erst durch die Kenntnis der Beziehung zwischen schlechter Belüftung des Mittelohres und Mittelohrerkrankungen Noch mehr stieg das Interesse an der Tubenfunktion naturgemaß mit der Entwicklung der modernen Mikrochirurgie des Ohres Es wurden

zahlreiche unterschiedliche, zum Teil sehr aufwendige Untersuchungstechniken für die Tubenfunktionsprufung entwickelt (8, 66, 68, 109, 170, 176)

Die Abhangigkeit der Ubertragungseigenschaften des Mittelohres vom Luftdruck innerhalb der Paukenhohle, also von der Tubenfunktion, wurden von mehreren Autoren in unterschiedlichen Experimenten bewiesen (34, 67, 98, 156, 191, 220, 239) Die Entstehung von Mittelohrergüssen nach kompletter Obturation der Tube untersuchten insbesondere Filsberg und Mitarb (67) und Paparella und Mitarb (180) Eine Ubersicht uber den heutigen Stand der Erkenntnisse uber die Funktion der Tuba auditiva gibt Feldmann (62, 63)

C. Problemstellung

Im ersten Teil dieser Arbeit sind die bekannten anatomischen Befunde am Mittelohr und die heute gültigen Theorien uber seine Funktion, soweit sie fur die Mikrochirurgie des Ohres Bedeutung haben, zusammengetragen worden Aus dieser Zusammenstellung laßt sich erkennen, daß insbesondere uber die Schwingungsform des Trommelfells keine einheitliche Auffassung besteht Nach der Theorie von v Bekesy schwingt das Trommelfell wie eine Klappe um die Rotationsachse der Gehorknochelchen Im Gegensatz dazu fand Tonndorf die über 100 Jahre alten Vorstellungen von Helmholtz über eine wesentlich kompliziertere Schwingungsform bestatigt Unter Ausbildung eines Hebelsystems, das in der v Bekesy-Theorie nicht vorliegt, ubertragt das Trommelfell Druckschwankungen auf die Gehorknochelchenkette

Fur die wiederherstellende Chirurgie der Membrana tympani resultieren aus den unterschiedlichen Grundlagentheorien über die Schwingungsform des Trommelfells differie- [illegible] Operationsprinzipien Anliegen der Mikrochirurgie des Ohres ist es, normale Verhaltnisse wieder herzustellen Um den Vorstellungen von v Bekesy zu entsprechen, würde man sich bemühen, dem Umbo gegenüber ein lockeres Gewebe zu schaffen, um hier dem „freien Klappenrand" ausreichende Bewegungsfreiheit zu geben Entsprechend den Befunden von Tonndorf ware es angezeigt, hier ein straffes Gewebe einzubringen um den normalen Elastizitatsverhaltnissen zu entsprechen

I SCHWINGUNGSVERHALTEN DES NORMALEN TROMMELFELLS

Wesentlich erschien das Schwingungsverhalten des normalen Trommelfells an Praparaten und auch in vivo am Menschen zu untersuchen Die Festlegung des normalen Schwingungsmusters mit seinen Variationen bei normalhorenden Patienten ist als unerlaßliche Voraussetzung fur die Beurteilung pathologischer Zustande anzusehen

II SCHWINGUNGSVERHALTEN DES ERKRANKTEN TROMMELFELLS

In weiteren Untersuchungen an menschlichen Felsenbeinpraparaten und am Patienten sollte der Einfluß von Störungen der Normalsituation auf das Schwingungsverhalten untersucht werden Als Hauptschadigungen waren dabei der Einfluß von Perforationen und Narbenbildungen nach Perforationen zu analysieren

1. Einfluß von Perforationen

Durch eine Perforationsbildung wird naturgemaß die wirksame Flache des Trommelfells verringert und gleichzeitig der Schallschutz fur den Paukenraum aufgehoben Durch beide Parameter laßt sich eine Schwerhörigkeit erklaren, wie sie klinisch bei Trommelfellperforationen haufig nachweisbar ist Unterschiedlich große Trommelfellperforationen in wechselnden Arealen dieser Membran konnen unterschiedliche Mittelohrschwerhorigkeiten hervorrufen Es ergab sich die Frage, inwieweit diese Befunde mit einer Anderung der Schwingungsform des Trommelfells erklart werden konnen

3. Einfluß von Narben

Auch bei abgeschlossenem Trommelfell sind nicht selten Schwerhorigkeiten nachweisbar, wenn ein Teil der Trommelfellmembran durch

eine atrophische oder schlaffe Narbe gebildet wird. Dieser Bereich des Trommelfells zeigt eine andere Konsistenz als der Rest der Pars tensa. Das Narbenareal legt sich in ungünstigen Fällen der medialen Wand des Mittelohres, dem Promontorium, an oder wölbt sich blasenförmig in den äußeren Gehörgang vor. Diese überblähten Bereiche erwecken schon bei statischer Beobachtung den Eindruck, daß sie zur Schallübertragung auf die Gehörknöchelchenkette nicht so geeignet sein können, wie eine intakte Pars tensa. Das Schwingungsverhalten solcher Narbenbildungen im Trommelfell am Menschen in vivo sollte untersucht werden.

III. SCHWINGUNGSVERHALTEN DES OPERIERTEN TROMMELFELLS

Mit zunehmender Leistungsfähigkeit der modernen Mikrochirurgie des Ohres und der Entwicklung einer funktionellen Chirurgie am Schalldrucktransformator Mittelohr hat die Zahl der operierten Patienten in den letzten Jahren zugenommen. In der Tübinger Universitäts-Hals-Nasen-Ohrenklinik wurden in den letzten 5 Jahren über 6000 mikrochirurgische Eingriffe am Ohr durchgeführt. Bei diesen Operationen wird das Trommelfell partiell oder komplett rekonstruiert oder es wird zur Verbesserung des Einblicks in den Mittelohrraum zum Teil aus seiner Verankerung im Limbus osseus herausgelöst und wieder zurückverlagert. In Abhängigkeit von der Größe des Trommelfelldefektes und auch von der Operationstechnik sowie einer Reihe anderer möglicher postoperativer Komplikationen erreicht das Hörvermögen nicht immer einen Optimalwert. Die Beobachtung des Trommelfells im postoperativen Verlauf legt die Vermutung nahe, daß unterschiedliche Abheilungzustände zu einer unterschiedlichen Schwingungspotenz bei sonst reizloser kompletter Abheilung führen können. Die Korrelation des postoperativen Trommelfellschwingungsbildes mit den Operationsdaten und so der Versuch einer Optimierung der Trommelfellplastik sollte versucht werden.

D. Methoden

I ALLGEMEINES

Methoden zur Beobachtung der Bewegung des Trommelfells sind Weiterentwicklungen der Techniken, die zur Beobachtung des Trommelfells in Ruhelage, also zur Erkennung pathologischer Veranderungen entwickelt wurden Chauliac erwähnte im 14 Jahrhundert dafür zum erstenmal einen Ohrenspiegel Bis dahin und auch spater noch wurde das Trommelfell im einfallenden Sonnenlicht betrachtet (25) Um die Mitte des vorigen Jahrhunderts wurde insbesondere von Wilde die regelmaßige Verwendung eines Ohrtrichters zur Inspektion des Trommelfells propagiert 1841 und 1855 erfanden Hofmann und v Trolsch unabhangig voneinander den perforierten Hohlspiegel zur Beleuchtung des Ohres *Den Entwicklungsweg bis zum heuti* gen koachsial beleuchteten Ohroperationsmikroskop und notwendiger Zusatzteile für die Registrierung von Befunden schildern Thullen (…1) und Lundborg (153)

1879 bemuhte sich Bosanquet (30) um die Einrichtung akustischer Labors und beschaf tigte sich mit der Frage, wie man Schwin gungen z B von Stimmgabeln ausmessen könne Vorher hatten Mach und Kessel an Felsenbeinpraparaten und an Modellen Untersuchungen uber die Schwingungsform des Trommelfells und der Gehorknöchelchen durchgeführt (156, 157 158) Beobachtungen am lebenden Menschen mit an einer Mauer fest fixiertem Kopf versuchte Waar 1923 (238) Er konnte unter monokularer mikroskopischer Betrachtung des Trommelfells bei 30 bis 100-facher Vergroßerung nach Beschallung mit Stimmgabeln oder lauten Knallen keine Bewegung des Trommelfells oder Hammergriffs feststellen, es gelang ihm aber bei 2 Patienten mit willkurlicher Kontraktionsmoglichkeit fur den M tensor tympani eine Bewegung des Umbo im Trommelfell zu beobachten

Im folgenden werden die bisher verwendeten Methoden zur Analyse von Trommelfell und Gehorknochelchenbewegungen be*schrieben*

1. Endpunktmessungen

Die erste klinisch leicht anwendbare Methode zur Uberprufung der Beweglichkeit des Trom melfells entwickelte Siegle 1864 (zit n 191) Es handelt sich um einen nach außen durch eine schragstehende Glasplatte abgeschlosse nen Ohrtrichter, in den durch Ansatz eines Gummiballons ein Uber- und Unterdruck erzeugt werden kann Insbesondere, wenn die abdeckende *Glasplatte* als *Lupe ausgebildet* ist, lassen sich Exkursionen des Trommelfells und des Hammergriffes beobachten Die Am plitude der Auslenkung kann abgeschatzt wer den und es laßt sich erkennen, welche Areale des Trommelfells den Druckschwankungen zu folgen vermogen

Frencker (71) versuchte 1939 die Befunde, die mit dem Siegle Trichter beobachtet wer den konnen, über eine Kamera zu fixieren und spater zu analysieren Er erzeugte analog der pneumatischen Lupe Unter- und Uberdruck im außeren Gehorgang und filmte bzw fotografierte die dadurch entstehenden Positions anderungen des Trommelfells und des Ham mergriffes Seine Beobachtungen ließen die Aussage zu, daß der Hammergriff sich parallel zur Normalposition ein und auswarts bewe gen kann, eine Tatsache, die mit der Bildung einer festen Rotationsachse durch die Aufhangebander nicht voll vereinbar erschien Ahnliches beobachteten auch Guelke und

Keen (87) sowie Tonndorf und Khanna (231) mit anderen Techniken

2. Sondenverfahren

a *Mechanische Sonden*

Politzer (191) war der erste, der 1861 an menschlichen Felsenbeinpräparaten durch Aufkleben von 10 bis 12 cm langen Glassonden auf Hammer und Amboß die Schwingungsvorgänge im Mittelohr zu analysieren versuchte Durch die langen, aufgeklebten Hebel wurden die Bewegungen innerhalb der Gehörknöchelchenkette erheblich vergrößert Bei Beschallung mit niederfrequenten Sinustonen oder Tongemischen ließen sich den Schallreizen analoge Schwingungsbilder an der Hebelspitze registrieren Die erhebliche Belastung des Mittelohrapparates durch Anwendung dieser mechanischen Sonden führte zu einer nicht genau kalkulierbaren Veranderung der Schwingungsform Den Effekt der Massenbelastung untersuchte Lüscher (34, 154) Verfeinerte mechanische Sondenverfahren gaben Frencker (72) und Wilska (251) an

b *Kapazitative Sonden*

Kapazitative Sonden, die die Anderung eines elektromagnetischen Feldes in Abhängigkeit von der Distanz der Sondenspitze zum Meßobjekt als Meßsignal verwenden, führte v Bekésy (13) in die Ohrphysiologie ein (11, 12, 17) Mit diesen Meßverfahren gelang zum erstenmal die Analyse von Schwingungsvorgangen bei niedrigen Schalldrucken im Bereich der Horschwelle Die Messungen wurden an menschlichen Felsenbeinpraparaten vorgenommen Die Ergebnisse wurden durch evtl postmortale Veranderungen, nicht aber durch zusatzliche Belastungen des auszumessenden, schwingenden System beeinflußt

Ein Nachteil der Methode besteht darin, daß das Meßareal punktförmig abgetastet werden muß so daß eine synchrone Analyse der auszumessenden Flache nicht moglich ist

Perlmann versuchte in ahnlicher Weise nach Aufkleben von kleinen Metallplattchen auf das Trommelfell, die Schwingungsvorgänge in diesem Bereich zu analysieren Er nahm dadurch mögliche Veranderungen durch Massenbelastung des Untersuchungsobjektes in Kauf (182)

c *Optische Sonden*

Ähnlich wie die Änderung des elektromagnetischen Feldes zwischen Meßobjekt und Sonde bei Verwendung kapazitativer Verfahren läßt sich die Änderung der Lichtstärke in Abhängigkeit von der Distanz zwischen einer Lichtquelle und dem Objekt bei Sondenverfahren als Meßsignal verwerten Brask (32) führte einen Lichtleiter in den Gehörgang ein, beleuchtete damit kleine Areale des Trommelfells und bestimmte durch eine geeignete Lichtmeßanlage bei Beschallung des Trommelfells die Distanzänderung zwischen Leuchtquelle und Trommelfelloberfläche Ähnlich wie bei der Verwendung kapazitativer Sonden kann es sich auch bei diesem Verfahren nur um ein punktförmiges Abtasten des Meßfeldes handeln

3. Reflektionsbestimmungen mit aufgeklebten Spiegeln

Nach Untersuchungen uber die Topographie des Mittelohres versuchten Mach und Kessel (157) 1875 die Bewegungsvorgange im Mittelohr besser zu analysieren als es mit mechanischen Sonden moglich gewesen war Sie verminderten die Massenbelastung und damit die instrumentell bedingten Änderungen der normalen Schwingungsform durch Aufkleben lediglich kleinster versilberter Glaspartikel auf den Hammergriff Ein von diesen Spiegelchen reflektierter Lichtstrahl wurde im Abstand von 4 m ausgemessen und gab Hinweise auf die Bewegungsform der Gehorknochelchen (157) Mit ahnlicher Technik untersuchte Wada (239), die Schwingungseigenschaften des Mittelohres bei Amphibien, Reptilien, Vogeln und Saugetieren Er konnte an Vogeltrommelfellen uber die Einwarts Auswartsbewegung hinaus an einzelnen Punkten auch

Bewegungen innerhalb der Trommelfellebenen wahrscheinlich machen (239) Link (150) klebte kleine Spiegelchen auf die Gehorknochelchen und auf die Membran des runden Fensters an menschlichen Felsenbeinpraparaten und konnte so Differenzen in den Schwingungsamplituden zwischen den Gehorknochelchen und der Membrana secundaria feststellen

4. Interferometrie – Laser-Holographie

Schatz (204) und Powell (193) untersuchten an Modellen, die Ausbildung von Schwingungsmustern in kunstlichen, kreisförmigen Membranen Als Meßsignal dienten bei entsprechender Beleuchtung unterschiedlich stark ausgeprägte, ringförmige Interferenzlinien, die bei Auslenkung im Bereich der Lichtwellenlange entstehen

Khanna und Tonndorf (134, 135, 136, 226, 227, 228, 230) entwickelten unter Verwendung eines modernen Beleuchtungssystems mit koharentem Licht, dem Laser, ein Meßinstrument zum Bestimmen submikroskopischer Schwingungsvorgange in biologischen Objekten Es gelang ihnen damit, Schwingungsamplituden von 3×10^{-10} cm zwischen 20 und 20 000 Hz zu analysieren In Weiterfuhrung ieser Arbeiten unter Zuhilfenahme der zeitich gemittelten Holographie gelang es den gleichen Autoren, Schwingungsmuster von Trommelfellen auch bei niedrigen Schalldrucken im Bereich der Horschwelle zu bestimmen Als besonderer Vorteil der Methode ist anzusehen, daß es mit ihrer Hilfe gelingt, synchron das gesamte Trommelfell zu untersuchen, also im Gegensatz zu den Sondenverfahren oder der Schwingungsanalyse mit aufgeklebten Spiegeln, nicht nacheinander Einzelpunkte zu messen, sondern in jedem Moment das Schwingungsbild des gesamten Meßfeldes festhalten zu können Als nachteilig erweist sich der große instrumentelle Aufwand und die Tatsache, daß ein Einblicktrichter von mindestens 45 Grad zum Meßfeld hin vorliegen muß, so daß Aufnahmen des Trommelfelles durch einen intakten Gehorgang am Menschen nicht moglich sind Eine besondere experimentelle Schwierigkeit besteht darin, daß die Distanz zwischen Meßgerat und Meßobjekt sich nur innerhalb 1/4 von einer Lichtwellenlange verandern darf

Untersuchungen mit der Laser-Holographie an Katzenohren und menschlichen Felsenbeinpraparaten zeigten ein anderes Schwingungsbild des Trommelfelles als es mit Sondenverfahren von v Békesy (17) wahrscheinlich gemacht worden war

Trotz der Moglichkeit, das Schwingungsbild der gesamten Trommelfellflache synchron ausmessen zu konnen, gelingt es mit der Laser-Holographie nicht, Horizontalbewegungen, also Veranderungen in der Ebene des Trommelfells nachzuweisen Ortsvektoren sind nicht darstellbar Bei zeitlicher Mitteilung ergeben sich Interferenzlinien nur in Einblickrichtung

5. Mossbauer-Effekt

Gilat und Mitarb (80) versuchten 1967 unter Ausnutzung des Mossbauer-Effektes, die sub mikroskopischen Bewegungsvorgange am Ohr zu messen

Die Untersuchungsmethode benutzt die Schallschnelle der zu analysierenden Objekte als Meßsignal, das sich damit bei zunehmender Frequenz um 6 dB/Oktave verkleinert Eine kleine ^{57}Cobalt-Strahlenquelle wird z B auf den Hammergriff aufgeklebt Durch radioaktiven Zerfall entsteht ^{57}Eisen, bei dessen weiterem Zerfall eine Gammastrahlung resultiert, die zur Messung verwendet wird Da sich der Ausgangsort dieser Gammastrahlung bewegt, entstehen bei Anwendung der Mossbauer-Methodik im Vergleich zu einer festen gleichen Strahlenquelle ein meßbares Strahlungsdifferenzbild, aus dem sich die Schnelle der bewegten Strahlungsquelle und damit die Amplitude errechnen laßt (123) Ausfuhrliche Messungen an unterschiedlichen Tierspezies (121, 122, 123) durch Johnstone und Mitarb zeigten, daß Messungen bis in den Bereich von

40000 Hz mit dieser Methode möglich sind Im Gegensatz zur Untersuchung mit der Laser-Holographie lassen sich mit dieser Technik nur Einzelpunkte nacheinander ausmessen Eine Synopsis der Trommelfellbewegung in jedem Moment ist nicht moglich

II STROBOSKOPIE

Wenige Jahre nach den grundlegenden Arbeiten vom Helmholtz (100) uber die Mittelohrphysiologie versuchten Mach und Kessel (158), die Bewegungsvorgange innerhalb des Mittelohres zu analysieren Sie verwendeten eine stroboskopische Technik, um die mit dem Auge nicht wahrnehmbaren Schwingungsvorgänge am Mittelohr sichtbar zu machen Mit einer Galvanischen Unterbrechungsgabel, die mit 256 Hz das auf das Meßfeld fallende Sonnenlichtbundel unterbrach, wurden definierte Lichtimpulse erzeugt Die Anregung der Schwingung erfolgte durch eine Pfeife von ebenfalls 256 Schwingungen/Sekunde die sich aber gegenuber dieser Grundfrequenz gering verstimmen ließ Es gelang auf diese Weise einen langsamen Bewegungseindruck der Schwingungsvorgänge zu bekommen

1. Prinzip der Stroboskopie

Bei der Stroboskopie handelt es sich um ein Verfahren, Schwingungsablaufe, die mit dem Auge nicht verfolgt werden konnen, so lang sam erscheinen zu lassen, daß die Beobachtung moglich wird Die schnelle, optische nicht differenzierbare Oszillation wird dabei in ihrer Frequenz ohne Anderung der Amplitu den durch einen speziellen Beleuchtungseffekt scheinbar so weit verlangsamt daß das menschliche Auge dem Bewegungsablauf zu folgen vermag Durch konsekutive kurzfristige Beleuchtung aufeinanderfolgender Phasenabschnitte einer durchlaufenden Wellenbewegung wird die zugrundeliegende hochfrequente Schwingung als ein langsamer Schwingungs vorgang erkennbar Das Prinzip wurde von Stampfer (1832 (212)) entdeckt

2. Technische Möglichkeiten

Um die Stroboskopie zu verwirklichen sind außer der schon genannten Lichtstrahlunterbrechungstechnik von Mach und Kessel (158) weitere technische Moglichkeiten gefunden worden

a *Elektronenblitz*

Die große Verbreitung im Hals Nasen Ohren bereich hat das Stroboskop in der Laryngologie zur Analyse von Stimmbildungsstorungen gefunden Die Beleuchtung der schwingenden Stimmbänder wird durch ein Elektronenblitzsystem vorgenommen, das sich über ein Mikrophon steuern laßt Uber das Mikrophon wird der Ton aufgenommen, der zum Unter suchungszeitpunkt von den Stimmbandern erzeugt wird Wird die jeweilig gleiche Phase der Stimmbandschwingung beleuchtet, so ergibt sich ein statischer Befund Verschieben sich die Beleuchtungsfelder innerhalb der Welle von Beleuchtungsblitz zu Beleuchtungsblitz geringfügig gegenüber der auslösenden Schwingung des Kehlkopfes, so erscheint die Kehlkopfschwingung langsam Die Differenz zwischen Beleuchtungsfrequenz und Schwin gungsfrequenz des Kehlkopfes ergibt die Geschwindigkeit des optischen Schwingungseindruckes

Basierend auf diesem Larynxstroboskop sind Elektronenblitzröhren auch für die mikroskopische Stroboskopie entwickelt worden Die im Handel erhaltlichen Geräte weisen den Nachteil auf, daß die Beleuchtung lediglich für die Betrachtung mit dem Auge ausreicht, nicht aber zur Aufnahme von Farbfilmen oder Farbfotos Mit der Möglichkeit, die Entladungsdauer von Elektronenblitzrohren elektronisch zu verlangern, wird sich hier in Zukunft auch die Moglichkeit der Dokumentation verbessern

b *Zeitlupenfilm — Hochgeschwindigkeitskamera*

Ein zeitlicher Dehnungseffekt ähnlich dem stroboskopischen Bild gelingt mit einer Hoch

geschwindigkeitskamera bei entsprechend heller Beleuchtung und empfindlichem Filmmaterial Schwingungsvorgange in ihrem naturlichen Ablauf konnen direkt fotographisch aufgenommen werden Bei langsamer Reproduktion des Filmes entsteht ein sog Zeitlupeneindruck, der es gestattet, schnelle Schwingungsvorgange langsam wiederzugeben, so daß ihre Beobachtung und Auswertung moglich wird

c *Lochscheibenstroboskop – Eigene Technik*

Ahnlich wie bei der Technik von Mach und Kessel (158) kann eine rotierende Lochscheibe, deren Locher jeweils den Beleuchtungsstrahl fur einen Moment auf das Objekt auftreffen lassen, eine stroboskopische Betrachtung von schnellschwingenden Bewegungsvorgangen ermoglichen Wird durch die Lochscheibe selbst zusatzlich auch der das Objekt in Schwingung versetzende Ton gesteuert, so ergibt sich auf mechanisch stabile Weise eine direkte Koppelung zwischen der Beleuchtungsfrequenz und der erregenden Tonfrequenz Ein langsam schwingendes Bild entsteht, wenn zum tongenerierenden Signal aus der Lochscheibe auf elektronischem Wege einige Tonimpulse/sec hinzuaddiert bzw subtrahiert werden Vorteile der Lochscheibenverwendung sind die mechanische Stabilitat und der geringe Raumbedarf

3 Versuche zur Verwendbarkeit am Ohr

Wir entschieden uns zur Untersuchung der Schwingungsvorgange am Trommelfell fur eine stroboskopische Technik Wegen des hohen technischen Aufwandes der zur Messung mit der Laser-Holographie oder dem Mössbauer-Effekt hatte getrieben werden mussen, wurde diese Methode von vornherein ausgeschlossen Es sollte eine klinisch in vivo am Menschen durchfuhrbare Untersuchungstechnik entwickelt werden

Als Vorversuch wurden das Stroboskop für die Larynxuntersuchung also ein Gerat das mit Elektronenblitzimpulsen das schwingende Meßfeld beleuchtet, verwendet Das Licht der Hochdruck-Xenonlampe wurde über Spiegel in den menschlichen Gehorgang geleitet Zur Betrachtung verwendeten wir ein Operationsmikroskop Die Beschallung erfolgte uber Radiolautsprecher

Schon bei diesem Vorversuch erwies sich die Xenonblitzlampe als zu lichtschwach fur die sichere Betrachtung des Trommelfells Eine fotographische Dokumentation z B in Form von Filmaufnahmen war nicht moglich Auch die Schallapplikation uber Raumlautsprecher erwies sich als ungenügend Die Schallintensitat war nicht so groß, daß Bewegungen am Trommelfell sichtbar gemacht werden konnten Die Belastung der Umgebung war trotzdem erheblich Aus diesem Grunde wurde fur die stroboskopische Untersuchung der Trommelfellschwingungen ein Stereo-Stroboskop neu entwickelt

4. Entwicklung eines Stereo-Mikrostroboskops

Die moderne Mikrochirurgie des Ohres ware nicht moglich gewesen ohne ein Operationsmikroskop, das bei koachsialer Beleuchtung die stereoskopische Beobachtung des Operationsfeldes im Mittelohr zulaßt Das heute fuhrende Instrument dieser Art ist das Ohroperationsmikroskop der Fa C Zeiss Als Zusatz zu diesem Operationsmikroskop wurde zur Analyse der Trommelfellschwingungen in Zusammenarbeit mit Dr Littmann, Dr Lang Dr Beste, Dr Leutwein und Dipl Physiker Muller ein Mikrostroboskop entwickelt

Entwicklungsziel war, ein Stroboskop zu erstellen, das ahnlich wie das Operationsmikroskop zwanglos auch auf das Mittelohr des Menschen in vivo eingestellt werden konnte, ohne am Patienten fixiert werden zu müssen, also unter Erhalt der freien Arbeitsmöglichkeiten wie beim Operationsmikroskop Um den technischen Aufwand gering zu halten, wurde auf das Prinzip des Lochscheibenstroboskops zuruckgegriffen

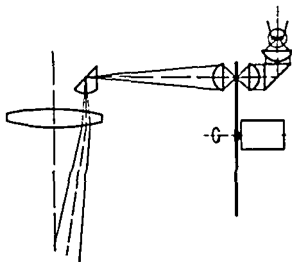


Abb 1 Die Lochscheibe unterbricht den Beleuchtungsstrahlengang in seinem Kreuzungspunkt

a *Technischer Aufbau*

Das Mikrostroboskop gliedert sich in 2 Hauptbestandteile, den Beleuchtungsteil des Gerates und die Vorrichtungen zur Applikation der Schallreize auf das Trommelfell

a 1 *Beleuchtungsteil* In den Strahlengang des normalen Operationsmikroskopes OPMI I wurde eine Lochscheibe so eingebaut, daß deren Öffnungen im Kreuzungspunkt des Beleuchtungsstrahles in Abhangigkeit von der Umdrehungsgeschwindigkeit der Lochscheibe für kurze Zeit Licht durchlassen Auf diese Weise wird erreicht, daß das Beleuchtungsfeld nicht durch die Öffnungen der Lochscheibe begrenzt wird, sondern seine normale Ausdehnung behalt (Abb 1)

Bei Prototypen hatte sich die Begrenzung des Leuchtfeldes durch die Große der Perforationen in der Lochscheibe als hinderlich erwiesen, da nicht immer das ganze Trommelfell ausgeleuchtet war

Der optische Aufbau des Operationsmikroskopes wurde bis auf das Anbringen der Lochscheibe nicht verandert Um die Helligkeit des Stroboskoplichtfeldes zu erhohen, konnte statt der normalen 50-W Gluhbirne eine 100-W-Halogen Lampe in das Mikroskopgehause eingebaut werden Die Beleuchtungsstarke reicht jetzt aus, um Fotographien und auch Farbfilme des sich bewegenden Trommelfelles herzustellen

Montiert ist die Lochscheibe auf einem kleinen Gleichstrommotor, dessen Umdrehungsgeschwindigkeit stufenlos geregelt werden kann Es ergibt sich damit die Möglichkeit, innerhalb eines breiten Frequenzbandes, nämlich zwischen 250 und 1 700 Hz, Lichtblitze zu erzeugen

Mit der Methode, die v Békésy (17) benutzte (kapazitative Sonden) waren oberhalb von etwa 2 000 Hz wesentliche Trommelfellschwingungen nicht mehr nachweisbar, also sehr klein Unsere Untersuchungen wurden deshalb auf den Frequenzbereich bis 1 700 Hz beschrankt

Das stroboskopisch, koaxial, parallaxenfrei beleuchtete Bild wird stereoskopisch unter Zwischenschaltung eines Vergrößerungswechslers in der Regel bei 16–25 facher Vergroßerung beobachtet Durch den Anschluß von Stereokameras oder einer Filmkamera laßt sich das Meßfeld simultan beobachten und fotographisch fixieren (Abb 2)

a 2 *Schallapplikation* Die Vorversuche hat ten ergeben, daß eine Schallzuführung über ein freies Schallfeld keine genügend hohe Reizintensitat zur Beobachtung von Trommel fellschwingungen unter den gegebenen stroboskopischen Verhältnissen zulaßt Es wurde deshalb ein Druckkammersystem entwickelt, das ahnlich der pneumatischen Ohrlupe von Siegle (zit n 191) Druckschwankungen auf das Trommelfell übertragen kann Dieses Druckkammersystem besteht aus einem Ohrtrichter und einem dicht auf diesem Ohrtrichter aufgebrachten abgeschrägten Ring, an dessen Seite ein Lautsprecher Schall abgibt Die schrage Deckplatte besteht aus Glas, so daß durch diese Glasplatte hindurch mit dem Mikroskop beobachtet werden kann (Abb 2)

Für die Tonerzeugung war es wichtig, eine stabile Korrelation zur Beleuchtungsfrequenz zu gewahrleisten Aus diesem Grunde wurde die Lochscheibe nicht nur fur die Erzeugung

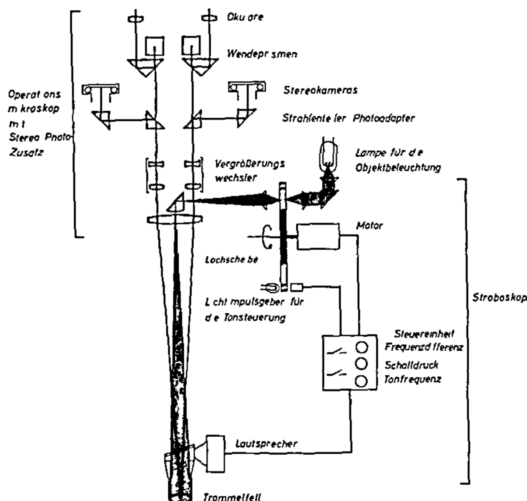


Abb 2 Schematische Darstellung des Stereo-M krostroboskopes

er Beleuchtungsblitze sondern auch zur Tongeneration verwendet Zusatzlich zu den Schlitzen für den Beleuchtungslichtstrahl wurden für jeden Beleuchtungsschlitz 32 feinere Perforationen angebracht (Abb 3) Uber diese feinen Schlitze wird ein Signal erzeugt das in einer entsprechend ausgerusteten Elektronik zur Tongeneration dient Durch Addition bzw Subtraktion von Impulsen laßt sich gegenuber der Beleuchtungsfrequenz eine in geringem Maße unterschiedliche Tonfrequenz variabel hervorrufen die uber den Lautsprecher dem Ohr zufließt Bei Ubereinstimmen von Lichtblitzfrequenz und Tonfrequenz scheint das Trommelfell stillzustehen Seine Exkursionen nehmen an Geschwindigkeit mit Zunahme der Differenz zwischen beiden Frequenzen scheinbar ebenfalls zu Eine besondere Schalttechnik ermoglicht es die Lichtblitzzufuhr und die Schallsignalphase so zu koordinieren daß an jedem beliebigen Punkt einer Trommelfellvibrationswelle Übereinstimmung von Beleuchtungs und Beschallungsfrequenz hergestellt werden kann so daß das Trommelfell bzw jedes Meßobjekt in jeder beliebigen Phase der Bewegung fixiert erscheint Auf diese Weise lassen sich z B Stereofotos bei maximaler Auswartswolbung und maximaler Einwartswolbung aufnehmen (Abb 3)

Die Untersuchungen erfolgen regelmaßig unter folgenden Bedingungen 200 mm Objektivbrennweite Vergroßerungswechslerstellung 16fach oder 25fach binokularer Geradtubus f=160 mm Okulare 20fach

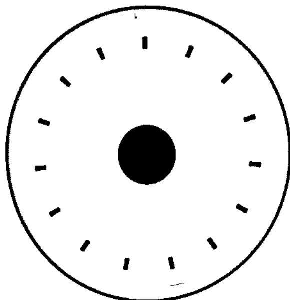


Abb 3 Lochscheibe

b *Eichung*

Aus den Herstellungsdaten des Mikrostroboskops und seiner Elektronik ließ sich direkt ein Frequenzwahler errechnen, uber den die jeweilige Beschallungsfrequenz eingestellt werden kann Nicht bekannt war, mit welchem Reinheitsgrad der zugefuhrte Schallreiz generiert wurde Insbesondere war zu klaren wie hoch der Obertonanteil bei der Erzeugung niederer Frequenzen sein wurde Aus diesem Grunde war eine Analyse der Schallreizzusammensetzung in Abhangigkeit von der vorgewahlten Frequenz unerlaßlich

Zur Beurteilung der Schwingungsamplituden am Trommelfell war es notwendig den jeweiligen Beschallungsdruck ebenfalls einstellbar am Steuergerat an einer willkurlichen Skala auszumessen Dies schien daruber hinaus noch erforderlich da bei sehr hohen Schalldrucken das Innenohr geschadigt werden kann Eine solche Gefährdung oder Schädigung sollte durch die durchzuführenden Untersuchungen nicht entstehen

Die Versuche zur Ausmessung des Mikrostroboskopes wurde im Landesamt für Arbeitsschutz und Arbeitsmedizin (Abtlg F, Larm und Erschutterung) in Karlsruhe in Zusammenarbeit mit Reg Gew Dir Dipl Phys Wolff, Dipl Phys Dr Gamber und Dipl Ing Mittag vorgenommen

Die Messungen erfolgten mit geeichten Larmmeßgeraten der Fa Bruel & Kjaer Der Schallapplikator mit Lautsprecher, glasbedecktem Schragzylinder und Ohrtrichter Nr 4 wurden in das „Artificial ear" Type 4152 Bruel & Kjaer, Serial No 388396 bei 2 ml Druckkammervolumen eingesetzt In dem kunstlichen Ohr wurde der Druck durch ein entsprechend geeichtes Druckmikrophon gemessen Das Normvolumen des kunstlichen Ohres von 6 ml war durch einen geeichten Einsatz auf 2 ml verkleinert worden Um einen dichten Abschluß zwischen Lautsprecher–Trichtersystem und dem kunstlichen Ohr zu erreichen, wurde das Testobjekt durch Zwischenschaltung einer Plastilinfullung zum Niveauausgleich auf das künstliche Ohr aufgedruckt (Abb 4)

Die Frequenzwahl erfolgte, ebenso wie die Lautstarkenbestimmung, über die Stroboskopelektronik

Zusatzlich wurde mit einem Transistorvoltmeter (Philips Multimeter, PM 2411 (944802411001) die Spannung des Lautsprecherstromes kontrolliert

Die Abgleichung der einzelnen Meßgerate

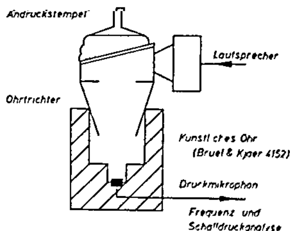


Abb 4 Lautsprechercharakteristik Meßanordnung am kunstlichen Ohr

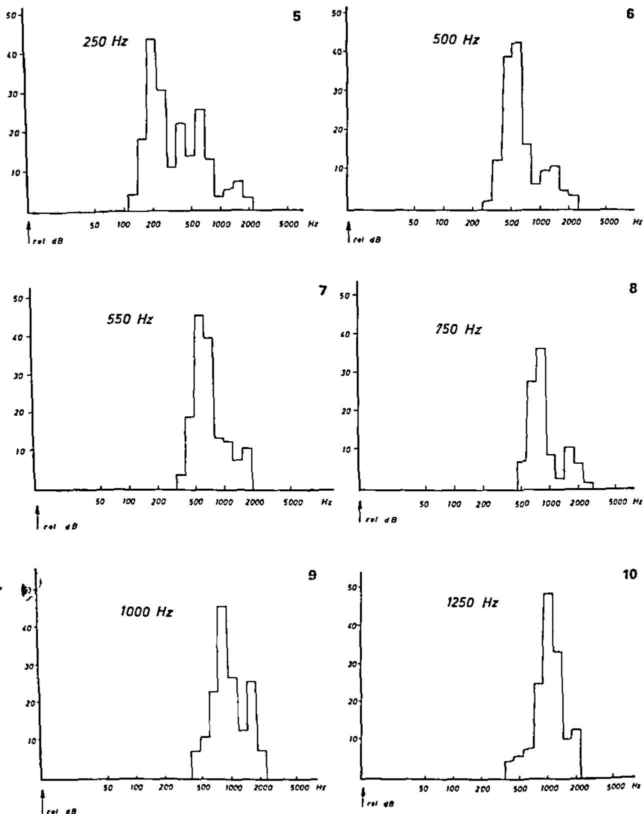
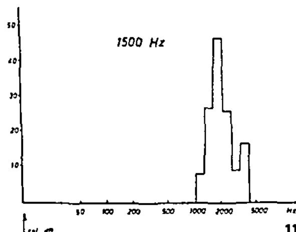


Abb 5–10 Schalldruck-(Ordinate) und Frequenzanalyse (Abszisse) bei unterschiedlicher Frequenzvorwahl



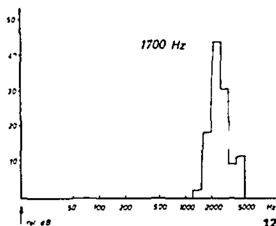


Abb 11 u 12 Schalldruck-(Ordinate) und Frequenzanalyse (Abszisse) bei unterschiedlicher Frequenzvorwahl

und des Meßplatzes erfolgte mit 124 dB bei 250 Hz Die Ausmessung der Lautstarke wurde uber einen Echtzeiterzanalysator (Brüel & Kjaer 3347) mit Kontrol und display unit (Type 4710) und Frequency analyser (Type 2130) vorgenommen

Die Frequenzanalyse der am Stroboskop vorgewahlten Frequenzen erfolgte uber die gleiche Meßeinrichtung Sie wurde in Relativwerten zusatzlich uber einen Level recorder (Bruel & Kjaer Type 2305) registriert

b 1 *Frequenzanalyse des Schallreizes* Die Analyse erfolgte bei einer Frequenzvorwahl für 250, 500, 550, 750, 1 000, 1 250, 1 500 und 1 700 Hz Aufgezeichnet wurden simultan in einem Relativmaßstab die Schalldrucke uber jeder Terzmittelfrequenz Am vorliegenden Meßplatz lassen sich Terzen mit folgenden Mittelfrequenzen ausmessen (Angaben in Hz)

12 5	50	200	800	3 150	12 500
16	63	250	1 000	4 000	16 000
20	80	315	1 250	5 000	20 000
25	100	400	1 600	6 300	25 000
31 5	125	500	2 000	8 000	31 500
40	160	650	2 500	10 000	40 000

Die Abb 5–12 sind Ubertragungen von Originalmeßstreifen

Die Breite der Kurven ist durch das unzulangliche Auflosungsvermogen der Meßgeräte mit der Aufzeichnung von Linienspektren in Terzbändern bedingt

Für eine 250 Hz Vorwahl trifft ein breites Linienspektrum als Schallreiz auf das Trommelfell, wobei der höchste Schalldruck im vorgewahlten Bereich liegt Für die hoheren Frequenzen zeigt das schallerzeugende System eine geringere Streuung Es lassen sich hohe Schalldruckspitzen im vorgewahlten Bereich beobachten Die Obertonintensitaten liegen wesentlich niedriger als das eigentliche Schallsignal

b 2 *Analyse des Beschallungsdruckes* Zur Bestimmung des Schalldruckes wurde in Abhangigkeit von der Schalldruckeinstellung an der Gerateskala gemessen und in einer weiteren Untersuchung in Abhangigkeit von der Spannung am Lautsprechereingang Die Messungen wurden jeweils mehrfach wiederholt und fuhrten konstant zu den gleichen Ergebnissen

Beim Abheben des schallzufuhrenden Lautsprecherohrtrichtersystems vom kunstlichen Ohr ließ sich erwartungsgemaß jeweils sofort ein Abfall des Schalldruckes im Meßsystem nachweisen Eine bekannte experimentelle Schwierigkeit ist die Abdichtung eines Druckkammersystems Am kunstlichen Ohr blieb der Schalldruck naturgemäß konstant

Verwendung eines mechanischen Andrucksystems

Es resultierte aus diesen Beobachtungen, daß bei Untersuchungen in vivo, in denen der Ohrtrichter den menschlichen Gehörgang nicht sicher abschließen kann, keine quantitative Angabe zur Trommelfellamplitude in Abhängigkeit vom Schalldruck möglich sein würde *Die gegebenen experimentellen Möglichkeiten ließen in vivo am Menschen nur Versuche im offenen System zu*

Die gefundenen Meßwerte werden im folgenden wiedergegeben

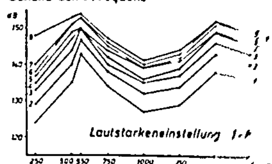
Meßwerte am künstlichen Ohr

Hz (Vorwahl)	Einstellung der Lautstärke	Spannung am Lautsprecher Volt	Unbewerteter Schalldruck dB
250	1	0 35	124
500	1	0 5	135
550	1	0 67	143
750	1	0 48	134
1 000	1	0 45	127
1 250	1	0 45	129
1 500	1	0 48	138
1 700	1	0 48	136
250	2	0 66	129
500	2	0 97	140
550	2	1 2	147
750	2	0 89	138
1 000	2	0 86	132
1 250	2	0 88	134
500	2	0 93	143
700	2	0 9	140
250	3	1 04	133
500	3	1,5	144
550	3	1 77	150
750	3	1 27	140
1 000	3	1 27	135
1 250	3	1 27	137
1 500	3	1 34	146
1 700	3	1 30	143
250	4	1 25	135
500	4	1 85	147
550	4	2 25	150
750	4	1 60	142
1 000	4	1 59	136
1 250	4	1 60	139
1 500	4	1 68	148
1 700	4	1 64	145
250	5	1 60	137
500	5	2 24	148
550	5	2 6	150
750	5	1 94	144
1 000	5	1 90	138
1 250	5	1 93	141
1 500	5	2 0	149
1 700	5	1 98	146
250	6	1 86	138
500	6	2 64	151
550	6	2 9	153
750	6	2 25	145
1 000	6	2 25	139
1 250	6	2 25	142
1 500	6	2 36	151
1 700	6	2 36	147
250	7	2 22	140
500	7	2 60	151
550	7	3 0	153
750	7	2 2	146
1 000	7	2 2	140
1 250	7	2 2	143
1 500	7	2 2	152
1 700	7	2 2	148
250	8	2 5	148
500	8	3 2	153
550	8	3 70	154
750	8	2 55	147
1 000	8	2 55	141
1 250	8	2 55	144
1 500	8	2 63	152
1 700	8	2 63	149

Meßwerte am künstlichen Ohr bei Konstanthaltung der Spannung am Lautsprechereingang

Hz (Vorwahl)	Volt am Lautsprecher	Unbewerteter Schalldruck dB	Lautstärkeneinstellung
250	0 5	127	1 2
500	0 5	134	0 9
550	0 5	139	0 6
750	0 5	134	1
1 000	0 5	127	1
1 250	0 5	130	1
1 500	0 5	138	0 9
1 700	0,5	135	1
250	1	133	2 8
500	1	141	1 9
550	1	145	1 5
750	1	138	2 1
1 000	1	132	2 1
1 250	1	135	2 1
1 500	1	144	2
1 700	1	140	2
250	1 5	137	4 4
500	1 5	145	3
550	1,5	149	2 2
750	1 5	141	3 7
1 000	1,5	136	3 8
1 250	1,5	135	2 1
1 500	1,5	144	
1 700	1 5	140	2
250	2 0	139	6 1
500	2 0	148	4 2

Hz (Vorwahl)	Volt am Lautsprecher	Unbewerteter Schalldruck dB	Lautstärkeneinstellung
550	2 0	150	3 6
750	2 0	143	5 1
1 000	2 0	138	5 2
1 250	2 0	141	5 2
1 500	2 0	149	5 0
1 700	2 0	146	5 1
250	2 5	141	7 8
500	2,5	151	5 1
550	2,5	152	4 8
750	2,5	145	6 6
1 000	2,5	139	6 6
1 250	2,5	142	6 6
1 500	2 5	151	6 2
1 700	2,5	148	6 4



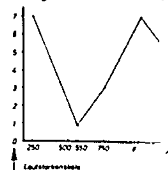


Abb 13 Lautsprec... Ohr) Die Line... wies sich insbes... beschrankt

Eine graphische Darstellung der tabellarischen Werte geben Abb 13 für das kunstliche Ohr und Abb 14 und 15 fur versuchsanaloge Bedingungen

Neben der instrumentellen Ausmessung des schallerzeugenden Systems wurde jeweils vor Applikation des Gehorgangstrichters in das Patientenohr vom normalhorenden Untersucher der eingestellte Schalldruck im eigenen Ohr auf Tolerierbarkeit uberpruft Audiogrammkontrollen auch nach Beschallungszeiten von 1 bis 2 Minuten wiesen keine Anderung der Innenohrleistung auf Reversible Schwellenverschiebungen insbesondere im Hochtonbereich sind erste Anzeichen einer Innenohrschadigung

Die Frage nach der Zumutbarkeit der Schallreize wurde zusatzlich durch Untersuchung der Arzte der Klinik beantwortet Bei allen ließ sich unter den zugeführten Schalldrucken die Schwingung des Trommelfells erkennen und beurteilen und jeder normalhorende Untersuchte empfand die zugefuhrten Lautstarken als laut aber tolerabel

5 Beurteilung der beobachteten Schwingungen

a *Stereobildauswertung*

Ahnlich der Erstellung von Landkarten aus stereoskopischen Landvermessungsfotographien die von ... men werden, ... Nahfotogrammetr... tung der Meßauf... nen Stereo Mik... technische Auf... der beobachtete... groß Die ...



Geschlossenes ...

Abb 14 ... analog den ...

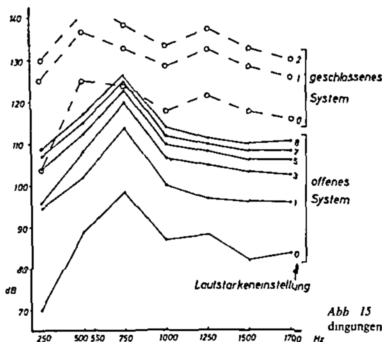


Abb 15 Lautsprechercharakteristik unter Versuchsbedingungen

von Bildpunkt zu Bildpunkt und fuhren zu einer dreidimensionalen Verzerrung Die Bewegungsunruhe bei der Beobachtung in vivo erschwert Vergleiche zwischen nacheinander aufgenommenen Bildpaaren Diese Fehler lassen sich nur durch den Einsatz von Computern mit komplizierten Programmen rechnerisch eliminieren Da für die Beurteilung von chwingungsbildern in vivo zusatzlich aus den ben genannten Grunden keine direkte Korre-tion zwischen Schalldruck und Amplitude der Schwingung aufgestellt werden kann, wurde die Fotogrammetrie im Nahebereich zur Auswertung unserer Trommelfellbilder nach Klarung der Moglichkeiten zuruckgestellt (Institut für Geodasie im Bauwesen, TH Stuttgart)

Weniger aufwendig als die Fotogrammetrie im Nahbereich ist die Auswertung von Stereobildern in einem relativen Maßstab, wobei Absolutgroßen der auftretenden Amplituden *nicht angegeben werden konnen* Nach Eichung des optischen Systems mit Objekten bekannter Oberflachenkonfiguration lassen sich solche Relativmessungen an Trommelfellbildern durchführen

b *Beobachtung und Beschreibung*

Die Beobachtung und Beschreibung der Bewegungsform des Trommelfells bei stereoskopischer Betrachtung unter dem entwickelten Mikrostroboskop hat den Vorteil, daß keine weiteren Meßgerate und Auswertverfahren zur Analyse notwendig sind Es besteht dabei der Nachteil, daß keine direkte Dokumentation in Form von Meßbildern, Tabellen oder Kurven entsteht

Zur Beurteilung der Reproduzierbarkeit beobachteter Schwingungsvorgange wurden an mehreren Tagen nacheinander gleiche Präparate untersucht Die Beobachtungen wurden in ein standardisiertes Trommelfellschema eingetragen Die Abweichungen zwischen den Einzelangaben variierten nur gering

c *Monoculares Filmen*

Um die zu beobachtenden Schwingungsvorgänge nicht nur einer geringen Zahl direkt mit*beobachtender Kollegen demonstrieren* zu können wurden Farbfilme uber einen der binocularen Strahlengange aufgenommen Der stereoskopische Effekt geht dabei naturgemäß verloren

E. Experimentelle und klinische Untersuchungen

Die vorliegenden Untersuchungen wurden vorgenommen an

62 menschlichen Ohren in vivo
36 menschlichen Felsenbeinpräparaten
20 normalen Kaninchenohren
16 operierten Kaninchenohren
5 normalen Rattenohren
5 operierten Rattenohren
4 normalen Meerschweinchenohren

In orientierenden Voruntersuchungen an Menschen in vivo ließ sich bei Normalfällen und an Patienten mit vernarbten Trommelfellen zeigen, daß die beschriebene Untersuchungstechnik geeignet war, Trommelfellschwingungen im gesamten Bereich der schwingenden Fläche erkennbar werden zu lassen. Auch für Normalhörende erschien die Lärmbelastigung durch das Setzen der Schallreize tolerabel. Bei Patienten mit einer Schwerhörigkeit reduzierte sich die subjektive Lärmempfindung naturgemäß entsprechend dem Ausmaß des Hörverlustes bei der jeweiligen Frequenz.

Zur Klärung des Schwingungsverhaltens des normalen Trommelfells wurden Versuche an menschlichen Felsenbeinpräparaten durchgeführt. Gegenüber normalen Testpersonen ergab sich hier die Möglichkeit, auch über längere Zeit mit hohen Drucken zu beschallen.

I. FELSENBEINPRÄPARATE

1. Entnahme

Menschliche Felsenbeinpräparate wurden im hiesigen Pathologischen Institut ohne Beschädigung der Strukturen des Mittel- und Innenohres 12 bis 24 Std. post mortem entnommen. Die Abtrennung erfolgte im Bereich der mittleren Schädelgrube in einer Linie vom Kiefergelenk zum Kanal der A. carotis interna und im Bereich der hinteren Schädelgrube entlang der Sutura petro-occipitalis.

2. Postmortale Veränderungen

Rückschlüsse von Versuchen am Leichenfelsenbein auf die Verhältnisse in vivo werden unzuverlässig, wenn postmortale Veränderungen die Schwingungsfähigkeit des Trommelfells beeinflussen.

Aus der Literatur ist bekannt, daß postmortale Veränderungen der Schwingungseigenschaft des Trommelfells insbesondere abhängig sind von der Art der Aufbewahrung. Es kommt durch Austrocknung schnell zu einer Zunahme der Steifigkeit des Trommelfells. Wird das Präparat in feuchter Atmosphäre aufbewahrt, so treten postmortale Veränderungen, die die Schwingungsfähigkeit verändern, nicht auf. Entsprechende ausführliche Untersuchungen führte 1923 Frank (69) durch. Er wies nach, daß die Elastizitätskoeffizienten des lebenden Ohres und die Spannung nicht wesentlich von den entsprechenden Größen des toten Ohres abweichen. Diese Ergebnisse werden ausdrücklich von v. Békésy, 1952 (14) nach erneuten Messungen mit verbesserter Technik bestätigt. Auch Guelke und Keen (86) sowie Kobrak (139) konnten keine die Schwingungsfähigkeit beeinträchtigenden Veränderungen bei entsprechender Lagerung beobachten. Die Untersuchungen von Zwislocki und Feldman (256), die im Gegensatz zu den vorgenannten Autoren Impedanzänderungen des Trommelfells post mortem feststellen konnten, beschreiben in ihren Arbeiten, daß sie die Gehörgänge vor der Untersuchung

Alkohol ausfüllten, um das Volumen des Gehorgangs bestimmen zu können Angaben zur Befeuchtung des Untersuchungspraparates finden sich nicht

Zur Uberprüfung der postmortalen Veränderungen in unseren Versuchen wurde bei einem Teil der Praparate die Austrocknung durch Befeuchtung verhindert Bei diesen Praparaten blieb das zu beobachtende Schwingungsmuster auch nach mehrfachem Tiefrieren des Praparates erhalten Konnte das Trommelfell aber austrocknen, so ergab sich in direkter Abhängigkeit von der Trocknungszeit eine zunehmende Minderung der Schwingungsamplituden

3 Aufbewahrung

Aus den Versuchen zur Bestimmung der postmortalen Veranderungen resultiert, daß die Bewahrung der Feuchtigkeit besonders wichtig für die Konstanterhaltung der Schwingungsvorgange ist Aus diesem Grunde wurden die entnommenen Felsenbeine regelmäßig nach Entnahme entweder sofort untersucht oder direkt auf −20°C tief gefroren Ahnlich wie es Perlmann (182) beschreibt, lassen sich bei dieser Aufbewahrungstechnik identische Versuchsergebnisse noch nach Monaten erzielen

4 Präparation

Unter stroboskopischer Beleuchtung sind Bewegungen, die rechtwinklig zum einfallenden Licht verlaufen, bei stereoskopischer und bei monocularer Betrachtungsweise gleichermaßen deutlich sichtbar, während Bewegungen parallel zur Lichteinfallsachse nur bei stereoskopischer Betrachtung in Erscheinung treten

Um auszuschließen daß der Einblickswinkel bei stereoskopischer Beobachtung einen wesentlichen Einfluß auf den optischen Eindruck hat, wurden 8 Felsenbeine so praparıert daß der Aufblick auf das Trommelfell aus beliebigen Winkeln möglich wurde Dazu entfernten wir den äußeren Gehörgang im Abstand von 1–2 mm vom Trommelfell und preßten das Trommelfellpräparat gegen eine perforierte Kunststoffscheibe Durch diese Einblicköffnung hindurch ließ es sich aus unterschiedlichen Richtungen mit wechselndem Einblickwinkel beobachten (Abb 16)

5 Versuchsanordnung

Der Gehorgangstrichter wurde zur Ausbildung eines Druckkammersystems durch Knetmasse gegen die Gehorgangswand abgedichtet Die Beschallung erfolgte mit Schalldrucken in der Großenordnung von 120 dB über den gesamten Frequenzbereich von 250 bis 1700 Hz in den Stufen 250 Hz, 500 Hz, 550 Hz, 750 Hz, 1000 Hz, 1250 Hz, 1500 Hz und 1700 Hz Durch Abdichtung des gesamten Felsenbeinpräparates ebenfalls mit Plastilin ließ sich der Luftdruck im Mittelohrbereich konstant halten In anderen Versuchen wurde dieser Abschluß nicht vorgenommen

Das Schwingungsverhalten zarter Narben, wie sie in vivo nicht selten zu beobachten sind wurde an 4 Felsenbeinpraparaten untersucht Die außere Epithelschicht des Trommelfells ließ sich abheben Die Tunica propria konnte ohne Setzen einer Perforation ausgedunnt werden Auf diese Weise entstanden in unterschiedlichen Arealen narbenähnliche Verhältnisse

An 8 Praparaten setzten wir Perforationen

6 Ergebnisse

a *Abhängigkeit des Schwingungsbildes von der Aufblickrichtung*

Unter dem Stroboskop mit monocularer Betrachtung werden Bewegungen vertikal zur Einfallsrichtung des Lichtes in ihrer natürlichen Ausdehnung solche in Richtung des einfallenden Lichtes aber nicht wahrgenommen Mit unserem Stereo-Stroboskop lassen sich Bewegungsvorgange auch in Beleuchtungsrichtung untersuchen

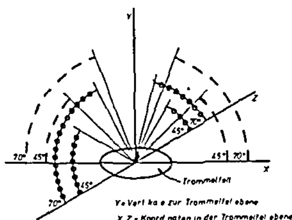


Abb. 16 Beobachtung des Trommelfells unter Aufblickwinkeln von 45° und 70°

Abstandsänderungen zwischen Objekt und Stereo-Mikroskop führen zu unterschiedlichen Abbildungswinkeln des Objektes. Diese Winkeldifferenzen sind sichtbar.

Für die Mikrochirurgie des Ohres gilt analoges. Nach entsprechender Vorübung lassen sich Distanzen unter dem Operationsmikroskop dreidimensional sehr genau abschätzen, unerläßlich z. B. zum Einpassen von Prothesen in Defekte innerhalb der Gehörknöchelchenkette.

b *Beobachtung des normalen Trommelfells*

Normale Felsenbeinpräparate wurden vor Austrocknung bewahrt und direkt nach Entnahme oder nach unterschiedlich langen Perioden des Tiefgefrierens untersucht. In Abhängigkeit von der Beschallungsfrequenz ließ sich bei gleichen Schalldrucken eine Abnahme der Amplituden, die im Trommelfell zu beobachten sind, vom Niederfrequenzbereich bis in den Hochtonfrequenzbereich nachweisen. Das Maximum der applizierbaren Frequenz unserer Anlage liegt bei 1700 Hz.

Für jede Frequenz wurde der Schalldruck unter Beobachtung des Trommelfells so lange gesteigert, bis in einem Areal die ersten Schwingungsvorgänge beobachtet werden konnten. Dieses Areal wurde in einer Trommelfellschablone zeichnerisch festgehalten. Mit langsamer Zunahme des Schalldruckes konnten die nachfolgenden Schwingungsareale eingezeichnet werden, bis das gesamte Trommelfell zu schwingen schien. Auf diese Weise ließ sich zeigen, welche Trommelfellareale die größten Amplituden aufweisen.

Unter Normalbedingungen sind dies die Trommelfellareale vor und hinter dem Hammergriff, während am unteren Trommelfellrand geringere Amplituden auftreten.

Zwischen dem Hammergriff und dem vorderen und hinteren Trommelfellrand liegen die Schwingungsmaxima etwa in der Mitte, was für eine gleichförmige Membran typisch ist. Die hintere Trommelfellhälfte schwingt wegen ihrer größeren Ausdehnung stärker als die vordere. Das dorsale Areal weist eine größere Distanz zwischen Anulus und Hammergriff auf als das vordere. Das Schwingungsverhalten war bei allen beobachteten Felsenbeinpräparaten identisch.

Zusätzlich zu dieser Bewegungsform der Trommelfellmembran, wie sie Helmholtz postuliert hat und wie Tonndorf und Khanna sie bestätigten, also im Gegensatz zu den Untersuchungen von v. Békésy, wurden in unserer Versuchsanordnung weitere Bewegungsvorgänge innerhalb des Trommelfelles sichtbar.

Betrachtet man Einzelpunkte in unterschiedlichen Arealen des Trommelfells, so entsprechen ihre Bewegungsvektoren wie bei jeder gleichförmigen Membran einer Vertikalen durch eine radiär liegende, örtliche Oberflächentangende (Abb. 17). Bei unterschiedlichen Elastizitäts- oder Massenverhältnissen, z. B. am Rand von Narben, kann es zu Abweichungen kommen.

In der Nähe des Hammergriffes jedoch kommt es zu tangentialen Verziehungen in Richtung auf den kurzen Hammerfortsatz. Diese Verziehungen der Trommelfelloberfläche sind abhängig von der Drehachse, um die der Hammergriff rotiert. Bei 2/3 der beobachteten Trommelfelle ließ sich das Achsenband als Rotationsachse annehmen, da der Drehpunkt des Hammergriffes etwa in der

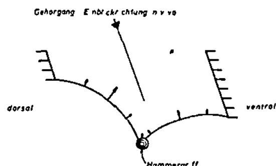


Abb 17 Ortsvektoren der Trommelfellbewegung Horizontalschnitt im distalen Hammergriffdrittel

Nahe des kurzen Hammerfortsatzes zu liegen schien In 1/3 der Falle zeigte sich eine gegen den Umbo verschobene Rotationsachse des Hammers z T in Abhangigkeit von der Frequenz Das Achsenband wurde mit der Gehorknochelchenkette nach einwarts gedruckt, wahrend sich der Umbo auswarts bewegte In einigen Fallen konnten wir beobachten, daß der Hammergriff keine Rotationsbewegung machte, sondern sich parallel zu seiner Langsachse einwarts bzw auswarts bewegte Dies ist als Folge der sehr hohen Amplituden anzusehen und entspricht den Vorstellungen von v Békésy (17)

c *Perforationen*

Durch Einbringen von schlitz- oder kreisförmigen Perforationen unterschiedlicher Große und an unterschiedlichen Arealen in das Trommelfell konnte sofort bestatigt werden, daß das Trommelfell keine wesentlichen elastischen Eigenschaften hat Insbesondere die schlitzförmigen Perforationen behalten ihre Schlitzform und verziehen sich nur in sehr geringem Maße (s a v Békesy (17) S 194)

Durch Einbringen von Perforationen wird das Prinzip der Trommelfellschwingung nicht verandert, d h bei sonst normalem Trommelfell bleibt das normale Schwingungsverhalten der phasengleichen Ein bzw Auswartsbewegung der Tunica propria erhalten Mit Zunahme der Perforationsgroße werden die Schwingungsamplituden verkleinert

d *Ausdünnung von Trommelfellbezirken*

Nach der Ausdünnung des Trommelfells in unterschiedlich großen Bereichen zeigte sich im Gegensatz zum Anlegen von Perforationen eine regelmaßig zu beobachtende Anderung des Schwingungsverhaltens Diese unterschiedlich stark verdunnten Trommelfellbereiche wiesen mit Zunahme der Ausdunnung eine Vergroßerung der zu beobachtenden Amplituden auf Blieben nur noch geringe Reste der Tunica propria erhalten, so resultierte in diesen Bereichen eine Abweichung in der Schwingungsphase Bewegt sich das gesamte Trommelfell in einer Richtung, so konnten diese Bereiche vollstandig gegenphasig zur anderen Seite schwingen Eine Austrocknung der Praparate wurde vermieden

e *Schwingungsverhalten bei defekter Gehörknochelchenkette*

Zur Uberprüfung dieser Frage wurde der Amboß aus der Gehorknochelchenkette entfernt Nach solchen Manipulationen blieb die außere Form und auch das Schwingungsverhalten des Trommelfells im Prinzip unverandert Die Amplituden wurden allerdings großer, d h schon bei geringen Schalldrucken ließen sich die typischen Schwingungsmuster erkennen

f *Narbig veranderte Trommelfelle*

Bei einem Teil der zur Verfügung stehenden Felsenbeinpraparate lag kein normales Trommelfell vor, sondern es fanden sich zarte Narben innerhalb der Membran Im Gegensatz zur normalen Bewegungsform mit phasengleicher Ein- bzw Auswartsschwingung unterschiedlichen Ausmaßes aller Areale der Pars tensa zeigten diese Narbenbezirke ein unterschiedliches Vibrationsverhalten In der Regel bewegten sie sich phasengleich mit den intakten Trommelfellanteilen im Niederfrequenzbereich zwischen 250 und 500 Hz Mit zunehmender Frequenz entstanden wellenförmige Bewegungsmuster, die haufig eine Phasenum

kehr gegenüber der intakten Umgebung aufwiesen Diese wellenförmige Bewegungsform, also eine unterschiedlich stark ausgeprägte Phasendifferenz gegenüber dem Normalverhalten war insbesondere abhängig von der Größe und von der Dicke der Narbe Erschien diese besonders dünn und zart, so waren die Wellenbewegungen früher und deutlich ausgeprägter zu erkennen als bei derben Narbenbildungen Kalkeinlagerungen innerhalb des Trommelfells storten den normalen Bewegungsablauf nicht Die genannten „Wanderwellen" sind nach Tonndorf (pers Mitteilung) als partielle stehende Wellen an Diskontinuitaten z B Narben aufzufassen

7. Diskussion der Ergebnisse

Beobachtungen an normalen und veränderten menschlichen Felsenbeinpräparaten stehen in weitgehender Übereinstimmung mit den Befunden von Tonndorf und Khanna (226 227, 228, 229, 230, 231) Ein normales Trommelfell schwingt in Phase, wobei die Maxima der Amplituden vor und hinter dem Hammergriff zu finden sind, während sich gegenuber dem Umbo geringere Auslenkungen zeigen

Zusatzlich zu den Befunden der genannten Autoren ließ sich bei einem Teil unserer Praparate eine Bewegung des Hammers erkennen, die mit der reinen Rotation um das Achsenband nicht vereinbar ist Die Ein und Auswärtsbewegung des kurzen Hammerfortsatzes weist auf eine analoge Verlagerung des Achsenbandes hin Möglicherweise hangt diese Bewegungsform ab von unterschiedlich stark entwickelten Aufhangebändern des Amboß

Als weitere bisher nicht bekannte Beobachtung kommt hinzu daß innerhalb der Trommelfelloberflache regelmäßig Verziehungen auftreten

Ähnlich wie Helmholtz (100) es gefordert hat aber nicht beobachten konnte, ließ sich mit unserer Untersuchungstechnik zeigen, daß die Bewegungsrichtung einzelner Punkte auf der Trommelfelloberflache eine senkrechte zu einer radiaren Oberflachentangente darstellt

Der Trommelfellkonus wird bei Auswarts bauchung des Trommelfells enger und bei Ein wartsbewegung weiter Eine echte Einwarts bauchung, also eine Umkehrung der Konus form, der sonst auswarts konvex ist nach ein warts konvex, konnten wir nicht beobachten Die Auswartskonvexitat des Konus bleibt bei allen Schwingungszustanden erhalten Dies gilt auch für sehr hohe Lautstarken, sodaß wir eine Schutzfunktion der Radiarfasern, die bei Einwartsbauchung den Hammer wieder auswartsziehen wurden (24) nicht bestatigen konnten Schon Helmholtz (100) hatte erkannt, daß das Trommelfell vor einer Ein wartswolbung seiner Trichterform zerreißen wurde

II TIERVERSUCHE AN KANINCHEN UND AN RATTEN

Nach horverbessernden, mikrochirurgischen Operationen am Menschen, z B nach dem Verschluß einer Trommelfellperforation bessert sich das Hörvermögen in der Regel so weit, daß eine mikrostroboskopische Untersuchung nur mit geringen Lautstarken moglich ist, da die uberwiegende Mehrheit der Patienten gegenuber stärkeren Schalldrucken sehr empfindlich reagiert Von Normalhorenden werden die gleichen Schalldrucke toleriert

Um das Schwingungsmuster des Trommelfells nach operativem Verschluß von Perforationen zu uberprufen und um insbesondere auch höhere Schalldrucke anwenden zu konnen, war es deshalb unerläßlich, Tierversuche durchzuführen Nach den günstigen Erfahrungen, die Steinbach und Hildmann (105, 106, 107, 214, 215) mit Tierversuchen an Kaninchen zur Klarung spezieller Fragen in der Mittelohrpathologie und Transplantationschirurgie am Ohre gemacht hatten, lag es nahe, auch für die vorliegende Fragestellung Kaninchen als Versuchstiere zu verwenden

Zusätzlich wurden Trommelfelloperationen an Ratten durchgeführt, um das Schwingungsverhalten bei einer weiteren Tierart zu beobachten

1. Operation

Die Kaninchen wurden in Evipan-Äthernarkose unter zusätzlich intramuscularer Gabe von Atropin am linken Ohr operiert (Wildstamme, Gewicht 3–4 kg, Grunfutter) Ausgewahlt wurden Kaninchen mit reizlosen äußeren Gehorgangen, insbesondere ohne ein Anzeichen einer Otitis externa

Nach retroauricularer Incision mit Vorklappen der Ohrmuschel gelingt eine ubersichtliche Darstellung des Trommelfells Teile der Trommelfellflache wurden excidiert und durch Unterfütterung mit einem Muskelfascienstuck oder durch Einlage von Homoiofascie wieder verschlossen In unterschiedlichen postoperativen Abstanden, aus Sicherheitsgründen bis zu 1 Jahr und 5 Monaten, wurden die Trommelfelle unter dem Mikrostroboskop untersucht Das rechte, nicht operierte Ohr diente jeweils als Normalfall, wahrend das linke die Beurteilung des postoperativen Schwingungsverhaltens zuließ Von 60 operierten Kaninchen konnten 37 zu den vorgeplanten Terminen analysiert werden 23 waren verstorben In der Halfte der Falle zeigte sich bei der Untersuchung eine ausgepragte Otitis externa, so daß das Trommelfell nicht beobachtet werden konnte Zur Stroboskopie verblieben 16 operierte und 20 normale Kaninchenohren Die Tiere waren keiner allgemeinen oder lokalen Antibiotica Behandlung unterworfen

2. Versuchsdauer

Die postoperativen Uberlebenszeiten wurden so gewahlt, daß mit Sicherheit endgültige Abheilungszustande der Operation zu beobachten waren Zusätzlich kann von den Langzeitversuchen mit fast 1½ jähriger postoperativer Beobachtungszeit angenommen werden, daß entsprechend den Erfahrungen bei der menschlichen Ohrchirurgie narbige Verziehungen in einem Endstadium vorlagen Fur den Menschen gilt als Regel, daß 1 Jahr post operationem keine weiteren narbigen Veränderungen auftreten

3. Versuchsanordnung zur Stroboskopie

Ahnlich wie bei der Untersuchung menschlicher Felsenbeinpraparate wurde für die Beobachtung des Tiertrommelfells ein entsprechend kleinerer Gehorgangstrichter verwendet Es wurde lediglich die Beobachtung durch den Gehorgang durchgeführt, da aus den Felsenbeinexperimenten die Frage der eventuellen optischen Verzerrung schon beantwortet war Die Beobachtung erfolgte unmittelbar nach Dekapitation

4. Ergebnisse

a *Stroboskopische Beobachtung*

Bei den normalen Ohren zeigte sich in vollkommener Ubereinstimmung mit den Beobachtungen an menschlichen Felsenbeinpraparaten ein phasengleiches Schwingen aller Areale des Trommelfells mit unterschiedlichen Amplituden, wobei die großten Amplituden vor und hinter dem Hammergriff auftraten und sich in Verlangerung des Hammers eine Amplitudenminderung erkennen ließ Die phasengleiche Schwingungsform bestand uber alle gemessenen Frequenzen von 250 bis 1700 Hz gleich Phasenunterschiede innerhalb der Trommelfellmembran, die sich als erstes in geringen Wellenformationen zeigen, wurden in keinem Fall beobachtet

Bei den operierten Ohren ergaben sich in Abhangigkeit von der Beschaffenheit der postoperativen Narben unterschiedliche Abweichungen vom normalen Schwingungsbild Derbe Narben mit gegenuber dem normalen Trommelfell starker Dickenzunahme wiesen regelmaßig eine geringere Schwingungsamplitude auf als die umgebenden normalen Trommelfellbezirke Im niederen Frequenzbereich zeigte sich eine phasengleiche Bewegungsform, wobei die Amplituden in der Narbe niedriger waren als im Restteil des Trommelfells Im hoheren Frequenzbereich, etwa ab 1000 Hz, konnten Wellenformationen beobachtet werden Sie entstehen, wenn unterschiedliche Areale des Trommelfells zu ver-

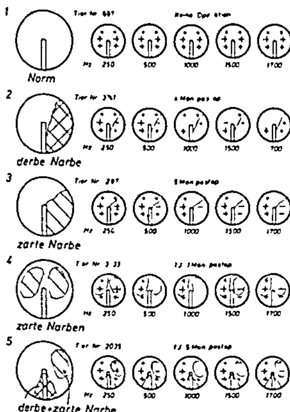


Abb 18 Schwingungsmuster des Kaninchentrommelfells nach Myringoplastik Die Auswärts (+) bzw Einwärtsbewegung (−) erfolgt entsprechend dem Ortsvektor (s Abb 17), Mittelstellung ( ) 1 Nicht operierte Seite 2 Vitale autologe Fascie 3 In Cialit konservierte Homoiofascie 4 Vitale autologe Fascie 5 In Cialit konservierte Homoiofascie

schiedenen Zeiten die maximale Auswärts- bzw Einwärtsbiegung erreichen Bei weiterer Zunahme der Frequenz bildeten sich Interferenzen heraus, so daß der optische Eindruck von Schaukelbewegungen entstand Zu gleichen Zeitpunkten waren manche Areale maximal einwärts (−) und andere Bereiche maximal auswärts gewölbt (+) (Abb 18)

Bei allen Abweichungen vom normalen Schwingungsverhalten des Trommelfells ließen sich ähnliche Phanomene beobachten Bei unterschiedlichen Frequenzen bildeten sich mit Zunahme der Frequenz erst Wellen und später Schaukelbewegungen aus Lagen besonders zarte Narbenbereiche innerhalb des Trommelfells, so ließ sich wiederholt eine Überhöhung der normalerweise auftretenden Amplituden im Niederfrequenzbereich beobachten Mit zunehmender Frequenz waren auch in solchen Fallen über das Stadium der Wellenbildung hinaus ausgeprägte Interferenzen zu beobachten (Abb 18)

b *Histologie*

Zur Überprüfung der Beobachtung unter dem Stroboskop wurden die operierten Kaninchen histologisch untersucht Es zeigte sich dabei eine Übereinstimmung der stroboskopisch gewonnenen Beschreibung mit dem histologischen Bild Eine als dick und derb erkannte *Narbe erwies sich auch im histologischen Bild* als eine Verdickung gegenüber dem normalen Trommelfell Entsprechendes zeigte sich auch bei zarten Narben Hier war die Bindegewebsschicht weniger stark ausgebildet als in der nicht operierten Umgebung Gleichzeitig ließ die histologische Untersuchung erkennen, daß die unterschiedlichen Schwingungsformen nicht etwa durch narbige Strangbildungen zwischen Trommelfell und medialer Paukenwand ausgelöst wurden

5. Diskussion der Ergebnisse

Die Untersuchung von normalen Kaninchen- und Rattentrommelfellen ergab die gleichen Befunde, wie sie schon an Trommelfellen menschlicher Felsenbeinpraparate erhoben worden waren In allen Fallen zeigte sich ein phasengleiches Schwingungsmuster aller Areale, wobei die Bereiche vor und hinter dem Hammergriff hohere Amplituden aufwiesen als die Gegend zwischen Umbo und Trommelfellrand in Verlangerung des Hammergriffes

Bei den *operierten Ohren zeigte sich in Abhängigkeit von der Anordnung der Narben innerhalb der Trommelfellfläche eine unterschiedlich stark ausgeprägte Abweichung vom normalen Schwingungsverhalten Es hellen sich als Ausdruck nicht mehr phasengleichen Schwingens einzelner Areale Wellenbewegungen und ausgeprägte Interferenzen* "

achten Einzelne Areale erreichten also zu unterschiedlichen Zeiten ihr Schwingungsmaximum oder zu gleichen Zeiten Schwingungsmaxima unterschiedlicher Richtung Solche Veranderungen konnten am operierten Trommelfell auch in Arealen beobachtet werden die keine Vernarbungszeichen aufwiesen so daß der Schluß zulassig ist daß durch die Narbenbildungen anderer Areale die physikalischen Parameter auch im restlichen noch normal erscheinenden Trommelfell so gestort waren daß ein phasengleiches Schwingen nicht mehr eintreten konnte (Abb 18 2)

Die Auslenkungen des Hammergriffes erschienen in solchen Fallen vermindert

Bewegungen innerhalb der Trommelfelloberflache *wie sie insbesondere im Bereich* des kurzen Hammerfortsatzes am menschlichen Felsenbeinpraparat regelmaßig nachweisbar waren konnten wir bei den Tierversuchen nicht beobachten Dies ist moglicherweise durch die wesentlich geringere Große des Trommelfells am Versuchstier bedingt Die am menschlichen Felsenbeinpraparat zu beobachtenden Amplitudenuberhohungen in Bereichen in denen die Tunica propria des Trommelfells ausgedunnt worden war fanden sich analog auch bei zarten Narbenbildungen

des operierten Versuchstierohres untersuchten Rattenohren zeigten ahn Schwingungsmuster

III TIERVERSUCHE AN MEERSCHWEINCHEN

Durch Versuche an Meerschweinchen sollte folgende Frage untersucht werden

Laßt sich mit dem neu entwickelten Stereo Mikrostroboskop neben der Schwingungsform des Trommelfells auch die Bewegungsform der Gehörknöchelchen und evtl der Perilymphe beobachten Wahrend die Amplituden am Trommelfell bei einem Beschallungsdruck von etwa 120 dB im Experiment am menschlichen Felsenbein oder am Kaninchentrommelfell so groß sind daß sie ohne Muhe beobachtet werden konnen war anzunehmen daß die Amplituden der Gehorknochelchen und insbesondere des ovalen und auch des runden Fensters wesentlich kleiner und moglicherweise auch bei hohen Schalldrucken zu klein für die Beurteilung mit dem beschriebenen Versuchsaufbau sein wurden

1 Versuchsanordnung

Durch eine Uberdosis Evipan i p getotete Meerschweinchen wurden so gelagert und prapariert daß man von dorsal in das Mittelohr blicken konnte Auf diesem Zugangsweg ließ sich das Trommelfell von innen erkennen Gleichzeitig waren Hammergriff Ende des *langen Amboßfortsatzes Stapeskopf also* das Amboß Stapesgelenk und in voller Ausdehnung die Membran des runden Fensters dargestellt Die Beschallung erfolgte mit dem beschriebenen Lautsprecher Ohrtrichtersystem durch den Gehorgang wahrend das Mikrostroboskop uber das eroffnete Mittelohr zentriert wurde Gemessen wurde bei variieren den Schalldrucken und Frequenzen analog zu den beschriebenen Untersuchungen

2 Ergebnisse

Wie bei den vorhergehenden Experimenten zeigte sich daß die normale Trommelfellmembran in phasengleicher Bewegung aller sichtbaren Abschnitte schwingt Dies fand sich im gesamten gemessenen Frequenzbereich Hammer Amboß und Stapeskopf zeigten für die niederen Schalldrucke unserer Meßanordnung (etwa 90 bis 100 dB) eine synchrone Bewegung Bei Einwartsbewegung des Stapes kam es im Frequenzbereich bis etwa 750 Hz zu einer phasengleichen Auswartsbewegung der Membran des runden Fensters Regelmaßig beobachteten wir bei hoheren Frequenzen phasendifferente Bewegungsmuster einzelner Teile der runden Fenstermembran wobei oberhalb von etwa 1 200 Hz mehrere Amplitudenmaxima nebeneinander auftraten Analoges fanden Khanna und Tonndorf (135)

Bei zunehmendem Schalldruck änderte sich dieses Schwingungsbild der runden Fenstermembran nicht. Es kam jedoch zu erheblichen Veränderungen innerhalb der Bewegungsform der Gehörknöchelchenkette, und zwar zu einer Dislokation im Amboß-Stapes-Gelenk. Bei Auswärtsbewegungen folgte der Stapeskopf dem Ende des langen Amboßfortsatzes nur noch unvollständig, wobei sich die Gelenkkapsel streckte. Bei weiterer Zunahme des Beschallungsdruckes riß diese Gelenkkapsel ein, so daß Amboß und Stapes voneinander getrennt wurden.

3. Diskussion der Ergebnisse

Die Untersuchung der Schwingungsverhältnisse im Mittelohr des Meerschweinchens, einem Standardversuchstier für die Analyse von Hörvorgängen, zeigte wie in den Versuchen an menschlichen Felsenbeinpräparaten oder an Kaninchen und Ratten ein phasengleiches Schwingen der normalen Trommelfellmembran und bei niedrigen Schalldrücken unabhängig von der gemessenen Frequenz eine synchrone Bewegung der Gehörknöchelchen. Die Ursache der Phasendifferenz, die auch bei niedrigen Schalldrücken oberhalb von 1000 Hz in der Membran des runden Fensters zu beobachten waren, wurde nicht erklärt. Sie zeigen aber, daß die Mechanik der Chochlea zu einer Modifikation der über die Stapesfußplatte eingegebenen Wellenbewegung führt.

Die Schutzfunktion der Gehörknöchelchenkette wurde deutlich in der Zerreißung des Amboß-Stapes-Gelenkes bei Schalldrücken über 120 dB. Eine Schutzfunktion im Hammer-Amboß-Gelenk läßt sich beim Meerschweinchen nicht beobachten, da dieses Gelenk regelmäßig durch eine Synchrondose überbrückt ist, so daß eine Verschiebung zwischen diesen beiden Gehörknöchelchen nicht auftritt. Vor der Zerreißung der Gelenkkapsel im Amboß-Stapes-Gelenk kommt es zu einer Dehnung und damit zu einer Minderung der Auswärtsbewegung des Stapes. Es folgt daraus, daß sich auf diese Weise die Gesamtamplitude der Stapes-Fußplatte reduziert, womit ein reversibler Schutzmechanismus erkennbar wird, während die Zerreißung naturgemäß eine irreversible Schädigung des Schalleitungsapparates bedeutet.

IV. UNTERSUCHUNGEN AM MENSCHEN IN VIVO

Nach den Beobachtungen der Trommelfellschwingungen am menschlichen Felsenbeinpräparat und in den Tierversuchen sowie nach Selbstversuchen zur Klärung der Frage, ob die applizierten Schalldrücke am Normalhörenden toleriert werden können, wurden Untersuchungen am Menschen in vivo vorgenommen.

Wie in den Vorversuchen beschrieben, wurde durch den Gehörgang beobachtet. Ein dichtes Abschließen der Ohrtrichterwand gegenüber der Gehörgangswand konnte dabei nicht erreicht werden, so daß die applizierten Schalldrücke nicht denen entsprachen, die am künstlichen Ohr gemessen wurden, sondern jenen bei nur inkompletter Abdichtung bzw. jenen mit freier Schallübertragung. Aus den entsprechenden Eichversuchen läßt sich der Schluß ziehen, daß Schalldrücke in der Größenordnung zwischen 90 und 120 dB angewendet wurden (Abb. 14, 15). Jede untersuchte Person bekam vor einem Test die Anweisung, den Beginn einer unangenehmen Lautheit sofort anzusagen.

1. Normales Trommelfell

Beim Betrachten des normalen Trommelfells eines Normalhörenden ergab sich regelmäßig folgendes Bild: Die Bereiche der Membran vor und hinter dem Hammergriff zeigten größere Amplitudenmaxima als die caudalen Trommelfellareale. Das gesamte Trommelfell wies regelmäßig ein Schwingungsmuster in Phase auf, d. h. alle Areale zeigten zur gleichen Zeit eine maximale Auswärts- bzw. Einwärtsschwingung. Auch angedeutete Phasendiffe

1 Norm — Hz 250, 500, 1000, 1500, 1700
2 zarte Narbe — Hz 250, 500, 1000, 1500, 1700
3 tympano meataler Winkel verdickt — Hz 250, 500, 1000, 1500, 1700
4 Perforation — Hz 250, 500, 1000, 1500, 1700
5 Homo o-Trommelfell 1 J postop — Hz 250, 500, 1000, 1500, 1700

Abb. 19. Schwingungsmuster des menschlichen Trommelfells in vivo. Symbole wie in Abb. 18.

renzen in Form von Wellenbildungen oder Interferenzen mit unterschiedlicher Richtung der Schwingungsmaxima zum gleichen Zeitpunkt *ließen sich bei Normalpersonen nicht beobachten* (Abb. 19. 1).

Die Normalpersonen applizierten Schalldrucke lagen zwischen 90 und 100 dB. Nur in Einzelfällen *mußte mit* 120 dB beschallt werden, um Schwingungen auf dem gesamten Trommelfell beobachten zu können. Auf eine komplette Abdichtung des Gehörganges gegenüber dem schallapplizierenden System verzichteten wir. Es läßt sich deshalb die Frage nicht entscheiden, ob differierende Grade der Trommelfellsteifheit oder unterschiedliche Schalldrucke am Trommelfell vorlagen. Zur Beobachtung annähernd gleicher Amplituden waren wechselnd starke Schallabstrahlungen vom Lautsprecher erforderlich.

Bei einzelnen Patienten mit einer einseitigen oder beidseitigen Destruktion des Innenohres, z. B. nach Lues, bei angeborener Schwerhörigkeit oder nach degenerativen Veränderungen im Innenohr bzw. am Hörnerven konnten auch normale Trommelfelle bei höheren Schalldrucken untersucht werden. Wieder wurden die Probanden gebeten, unangenehme Sensationen wie z. B. Schmerz sofort mitzuteilen. Auch unter diesen Versuchsbedingungen an der Grenze der Schmerzschwelle zeigte sich im Normfall ein phasengleiches Schwingungsverhalten aller Areale des Trommelfells. Die Schalldrucke konnten bei solchen Patienten z. T. *soweit erhöht werden*, daß Schwindelerscheinungen auftraten. Diese wurden früher geäußert als Schmerzgefühle.

Bei starker Schalldruckzufuhr ließ sich naturgemäß die Bewegung des Hammergriffes am besten beurteilen. Analog zu den Beobachtungen am menschlichen Felsenbeinpräparat zeigte sich keine konstante Drehachse der Bewegung, sondern diese war variabel. Oft schien sie in enger Beziehung zum Achsenband zu liegen. Wir beobachteten aber einige Patienten, bei denen das Minimum der Hammergriffbewegung etwa in dessen Mitte lag, so daß geschlossen werden muß, daß das gesamte Achsenband, sichtbar an der Bewegung des kurzen Hammerfortsatzes, bei den Schwingungsvorgängen einwärts und auswärtsverlagert wurde.

Bei einem Teil der Patienten wurden die Beobachtungen monocular farbig gefilmt.

2. Vernarbtes Trommelfell

Ausgedehnte schlaffe Narben im Trommelfell führen zu einer Minderung der Leistungsfähigkeit des Mittelohres (Abb. 20).

Dieses Phänomen ist klinisch nicht selten zu beobachten. Die Analyse der Schwingungsform in solchen Fällen zeigt, daß bei niederen Frequenzen der Bereich der schlaffen Narbe wesentlich höhere Amplituden aufweist als die umgebenden normalen Bereiche des Trommelfells (Abb. 19. 2). Der Bewegungsvorgang

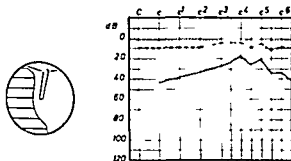


Abb 20 Schlaffe Narbe im Trommelfell

zwischen maximaler Einwärts- und Auswartsbewegung erfolgt in diesem Narbenbereich aber auch bei niederen Frequenzen nicht immer sinusförmig, sondern gelöst von der Bewegung der Umgebung klappend Im Zustand der maximalen Auswärts- bzw Einwartsbewegung verharrt die Narbe länger als die umgebenden Areale Die entgegengesetzte Maximalauslenkung wird durch eine schnellende Bewegung erreicht Es handelt sich um die Beobachtung von nichtlinearen Schwingungsformen im Sinne eines „peak clipping" Mit zunehmender Frequenz zeigen sich im Narbenbereich wellenförmige Bewegungsvorgange und bei unterschiedlichen Frequenzen Interferenzmuster, wobei zu gleichen Zeiten Schwingungsmaxima in unterschiedlicher Richtung ausgepragt sind Narbenstrange im cranialen Teil des Trommelfells etwa in Projektion auf das hintere Hammerband storen die normalen Bewegungsvorgange der Pars tensa des Trommelfells nicht Auch scheinbare Inhomogenitaten innerhalb der Tunica propria mit wabenartiger Struktur besonders zarter Trommelfelle fuhren nicht zu beobachtbaren Veranderungen der normalen Schwingungsform vorausgesetzt daß das gesamte Trommelfell homogen besonders zart strukturiert ist In diesen Fallen liegen die Schwingungsmaxima ebenfalls vor und hinter dem Hammergriff, wahrend sich ein Minimum der Schwingung im caudalen Trommelfellbereich findet

Bei tympanosklerotisch veranderten Trommelfellen mit ausgedehnten Kalkplatten vor und hinter dem Hammergriff und beweglichen Membrananteilen im caudalen Bereich der Membran zeigt sich, daß die den Limbus osseus erreichenden Kalkplatten auch bei den maximal applizierbaren Schalldrucken nicht vibrieren, daß aber die verbliebenen schwingungsfahigen Areale deutlich den Schallwellen folgen konnen In solchen Fällen resultiert eine Schalleitungsschwerhörigkeit

3. Perforiertes Trommelfell

An Patienten mit Perforationen des Trommelfells in unterschiedlichen Arealen zeigten sich gegenuber den Untersuchungen am menschlichen Felsenbeinpraparat keine Differenzen Es gelang an Patienten nicht immer, insbesondere nicht bei ausgedehnten Perforationen, eine Bewegung der Trommelfellreste oder des Hammergriffes zu beobachten Die notwendige Lautstarke lag im Schmerzbereich Bei kleinen Perforationen ohne umgebende Narbenbildungen wies das Schwingungsmuster ahnlich wie bei einem normalen Trommelfell ein phasengleiches Schwingungsverhalten der verbliebenen Areale auf

4 Zustand nach Myringoplastik

Bei einer Myringoplastik (20, 28 29, 162, 184, 186, 187, 253) wird der Trommelfelldefekt umschnitten, der verbliebene Trommelfellrest an der Unterseite angefrischt und mit einem Stuck Temporalisfascie unterlegt Diese Temporalisfascie wird durch zusatzlich angelegte winzige Perforationen im Trommelfellrand nach außen zipfelförmig ausgezogen und damit an der Unterseite fixiert Bei großeren Perforationen wird ein freies Hauttransplantat aus der vorderen Gehorgangswand zusatzlich auf die Fascie aufgelegt in der Weise, daß die Rander dieses Transplantates „Stoß an Stoß" (187) dem verbliebenen Trommelfellepithel der Außenflache anliegen Fur etwa 3 Wochen bleibt der Gehorgang mit einem antibiotika getrankten Gelatine Tampon verschlossen Nach 4 bis 6 Wochen laßt sich eine Abheilung

mit Verdünnung des operierten Trommelfellbereiches erkennen, und nach etwa 1/2 bis 1 Jahr ist ein endgültiger Vernarbungszustand eingetreten Das operierte Trommelfell weist in der Regel jetzt eine homogene Struktur auf, insbesondere dann, wenn nur kleine oder schlitzförmige Perforationen vorgelegen haben Hat eine ausgedehnte Defektbildung bestanden, so ergeben sich manchmal zarte Narbenfelder oder auch Bereiche, in denen Kalkeinlagerungen vorliegen Das Mittelohr ist regelmaßig normal beluftet Zeichen von Adhasionsstrangen zwischen operiertem Trommelfellbereich und medialer Paukenwand zeigen sich normalerweise nicht

Fur die stroboskopische Untersuchung wurden Patienten ausgewahlt, bei denen es zu einer reizlosen Abheilung nach Myringoplastik im beschriebenen Sinne gekommen war Es zeigte sich bei diesem Patienten, wenn nur eine kleine oder eine schlitzförmige traumatische Perforation vorgelegen hatte, regelmaßig ein normales Schwingungsbild ohne beobachtbare Wellenbildungen auch im hoheren Frequenzbereich und ohne andere Formen von Interferenzbildern

Hatte eine ausgedehnte Perforation von 1/3 bis zur Halfte der Trommelfellflache vorge- [illegible] so konnte dieses der Norm ent[illegible] Schwingungsverhalten auch beob[illegible]tet werden auch wenn die unregelmäßige Vascularisation der Trommelfellmembran noch auf die fruhere Operation hinwies Die Transparenz war dann in allen Bereichen annahernd in gleicher Weise gemindert so daß auf eine gleichmaßige Dicke der Membran geschlossen werden konnte Das der Norm analoge Schwingungsmuster wies auf eine der Norm angenaherte Steifheit aller Areale hin Unterschiede zeigten sich in solch gunstigen Fällen lediglich in der Große der Amplituden Diese waren nicht wie im Normalfall uber die Flache verteilt Die Maxima konnten sich verschieben, so daß zum Beispiel der vordere Teil der Membran ein etwas geringeres Maximum aufwies als der caudale Trommelfellbereich Die Amplitudenmaxima lagen aber zu gleichen Zeiten in der gleichen Richtung Wellenbewegungen wurden in diesen Fallen nicht beobachtet

Haufiger zeigte sich nach operativem Verschluß ausgedehnter Trommelfellperforationen jedoch ein Schwingungsmuster mit wellenförmigen Bewegungen, so daß auf beginnende Interferenzen geschlossen werden muß Die einzelnen Areale insbesondere die operierten Teile des Trommelfells erreichten ihr Schwingungsmaximum in einer Richtung zu anderen Zeiten als die Umgebung Ausgepragte Interferenzen mit Maxima der Trommelfellamplituden in unterschiedlicher Richtung zur gleichen Zeit fanden sich in jenen Fallen, die im operierten Bereich ausgedehnte zarte Narben aufwiesen Kalkeinlagerungen in Narbenfeldern änderten das Schwingungsbild im Trommelfell nicht, so lange sie den Trommelfellrand nicht erreichten

Bei inkompletter Ankoppelung eines rekonstruierten Trommelfelles an den Hammergriff, also bei einer Ablosung der Membran vom distalen Hammergriffdrittel, ließ sich regelmäßig nachweisen, daß das Trommelfell oberhalb des frei in die Pauke ragend, nicht angekoppelten Umbobereiches schwingen konnte Bei Frakturen des Hammergriffes wurde der distale Teil dieses Gehorknochelchens frei mit in die Trommelfellschwingung integriert Den funktionellen Umbo bildete das proximale Hammergriffende am Frakturspalt

5. *Zustand nach Operation an Trommelfell und Gehorknöchelchenkette*

Operationen an Trommelfell und Gehörknöchelchenkette sind erforderlich, wenn entzundliche Prozesse neben einer Trommelfellperforation eine Defektbildung innerhalb der Gehörknochelchenkette z B durch Arosion des langen Amboßfortsatzes hervorgerufen haben Wahrend desselben Eingriffes wird in solchen Fallen in der Regel die Trommelfellperforation in der oben beschriebenen Weise verschlossen und gleichzeitig durch Interposition eines konservierten Leichengehör-

knöchelchens oder durch Einbringen von Drahtbrücken der Defekt in der Gehörknöchelchenkette überbrückt (60, 96, 185, 186, 187, 203, 209, 253).

Die stroboskopische Untersuchung auf diese Weise operierter Patienten ist nicht immer so durchführbar, daß es gelingt, alle Areale des Trommelfells in ihrer Schwingungsform zu beobachten. Nach einer gelungenen Operation ist das Hörvermögen gegenüber dem präoperativen Zustand so verbessert, daß die Patienten durch die notwendigen Schalldrucke stark belästigt werden.

Bei unbefriedigendem postoperativem Ergebnis läßt die stroboskopische Untersuchung sich zwar mit höheren Schalldrucken durchführen. Die dann sichtbaren Bewegungsmuster werden aber mit Wahrscheinlichkeit auch von Adhäsionssträngen zwischen rekonstruiertem Trommelfell und medialer Paukenwand hervorgerufen. Bei Nachoperationen wegen unbefriedigenden postoperativen Hörvermögens zeigen sich solche fixierenden Bindegewebsstränge.

Von besonderem Interesse erschien das Schwingungsverhalten eines rekonstruierten Trommelfells, wenn die gesamte Gehörknöchelchenkette oder Hammer und Amboß durch die Implantation eines konservierten Leichenambosses wieder aufgebaut worden waren. Präoperativ besteht ein ausgedehnter subtotaler oder totaler Trommelfelldefekt. Postoperativ zeigt sich nach etwa 1/2 bis 1 Jahr eine zarte, rekonstruierte Trommelfellmembran, durch die der interponierte Amboß hindurchschimmert. In solchen Fällen mit annähernd normalem Hörvermögen läßt die stroboskopische Untersuchung des rekonstruierten Trommelfells Differenzen zum Normalen erkennen. Während normalerweise eine ausgeprägte Trichterform mit konvexer Auswärtsbauchung der Radiärfasern besteht, weisen die beschriebenen rekonstruierten Trommelfelle, bedingt durch die Operationstechnik, eher eine dem Vogeltrommelfell analoge Form auf. Der Bereich der Ankoppelung zwischen Trommelfell und Gehörknöchelchenkette, hier also zwischen dem rekonstruierten Trommelfell und dem interponierten Leichenamboß, ist prominent, während die übrigen Teile des Trommelfells einwärtskonvex gebogen sind. In günstigen Fällen mit optimalem Hörergebnis zeigt die gesamte rekonstruierte Trommelfellmembran ein Schwingungsmuster, das sich phasengleich bewegt. Derbe Narbenstränge, erkenntlich an ihrem weißlichen, undurchsichtigen Schimmer, vibrieren dabei deutlich weniger als zartere benachbarte Areale. Nur ausnahmsweise war dieses Schwingungsmuster auch in den höheren gemessenen Frequenzen, also oberhalb von 1500 Hz als phasengleiche Schwingung nachweisbar. Meistens zeigten sich mit zunehmender Frequenz wellenförmige Verformungen der rekonstruierten Trommelfellmembran als Zeichen einer Phasendifferenz. Die Bewegungen des äußersten Teils der rekonstruierten Gehörknöchelchenkette, also z. B. des interponierten Leichenambosses erschienen geringer als die des natürlichen Hammergriffes.

Bei inkompletter Ankoppelung des Trommelfells an die rekonstruierte Gehörknöchelchenkette, also in einer Situation, in der die Trommelfellmembran nicht die ganze laterale Oberfläche des interponierten Leichenambosses berührte, ließ sich die Schwingung des rekonstruierten Trommelfells bis zur Ansatzstelle hin verfolgen.

6 Homoio-Trommelfell

Bei einem totalen Trommelfelldefekt und sonst entzündungsfreier, reizloser Pauke ist es nicht selten indiziert, ein Leichentrommelfell nach entsprechender Vorbehandlung (Marquet (162)) zu transplantieren, um eine Abdeckung der Mittelohrräume zu erreichen. In einer 2. Sitzung kann die Gehörknöchelchenkette rekonstruiert werden (60).

Ähnlich wie bei der Verwendung von Temporalisfaszie ist die Einheilung des Transplantates nach etwa 1/2 bis 1 Jahr abgeschlossen. Durch Beobachtung an Patienten mit implantiertem Homoio-Trommelfell ließ sic

daß diese Transplantation bei kompletter Einheilung gute Voraussetzungen für die Rekonstruktion eines normalen Mittelohrmechanismus bietet In den untersuchten Fallen zeigte sich eine phasengleiche Schwingungsform des ganzen Trommelfells in Analogie zum Normalfall für die niederen und mittleren Frequenzen Für hohere Töne oberhalb 1200 Hz ließen sich Wellen als Zeichen der Ausbildung von Interferenzen zwischen einzelnen Trommelfellarealen nachweisen (Abb 19, 5) Deutlich bewegte sich der Hammergriff bei unseren Beobachtungen mit Insgesamt erschien die Form des Trommelfells, also die Ausbildung des normalerweise vorhandenen Trommelfelltrichters 1 Jahr post operationem flacher zu sein als bei dem normalen Mittelohr.

7. Ergebnisse

Die Beobachtungen an menschlichen Trommelfellen in vivo, nach Mittelohroperationen und auch bei Normalpersonen ohne Ohrerkrankungen in der Anamnese zeigen das gleiche Schwingungsverhalten des Trommelfells, wie es in den Versuchen an menschlichen Felsenbeinpraparaten und in den Tierversuchsserien beobachtet werden konnte Normalerweise schwingt die Membran phasengleich in allen Arealen (Abb 19, 1) Durch Narbenbildungen wird dieses Bewegungsmuster gestort, es treten ubermäßige Amplitudengrößen in zarten Narben (Abb 19 2) oder Amplitudenverkleinerungen bei der Ausbildung von narbigen Randwulsten z B im tympanomeatalen Winkel in Erscheinung (Abb 19, 3) Nach Operationen lassen sich als Zeichen einer Phasendifferenz zwischen einzelnen Bezirken des rekonstruierten Trommelfells wellenförmige Bewegungsvorgange beobachten In ungünstigen Situationen kommt es in Analogie zu den Tierversuchen zur Darstellung ausgepragter Interferenzen mit Amplitudenmaxima in unterschiedlichen Richtungen zum gleichen Zeitpunkt Sind größere Areale des Trommelfells durch z B einen entzündlichen Prozeß zerstört worden, so laßt sich die Form eines normalen Trommelfells durch eine mikrochirurgische Ohroperation in der Regel nur zum Teil wieder herstellen Die natürlicherweise vorhandene tiefe Trichterform flacht sich regelmaßig ab Lediglich wenn ein normaler Hammergriff noch zur Verfügung steht und eine der Norm entsprechende Ankoppelung des rekonstruierten Trommelfells an den Hammergriff gelingt, wird die ursprüngliche Trichterform annähernd wieder erreicht Ist auch der Hammer zu rekonstruieren, z B durch Einbringen eines Leichenambosses, so andert sich das Membranrelief Es resultiert, bedingt durch die Operationstechnik mit dem Ziel, eine stabile Gehorknochelchenkette zu rekonstruieren, eine Trommelfellform wie sie bei Vogeln verwirklicht ist Die Konusbildung eines solchen Trommelfells ist der normalen Form entgegengerichtet und insgesamt wesentlich flacher Ein auf diese Weise rekonstruierter Trommelfellmittelohrapparat zeigt phasengleiche Schwingungsvorgange im Nieder- und Mittelfrequenzbereich, fur höhere Frequenzen oberhalb 1200 Hz aber in den meisten Fallen Interferenzen, die als Wellen deutlich werden

F. Zusammenfassung der experimentellen und klinischen Befunde

Die Beobachtung von Trommelfellen in menschlichen Felsenbeinpräparaten, in Tierversuchen an Kaninchen, Ratten und Meerschweinchen sowie an Menschen in vivo, und zwar an normalen Trommelfellen und nach mikrochirurgischen Ohroperationen führt zu übereinstimmenden Ergebnissen

Ein normales Trommelfell zeigt ein phasengleiches Schwingungsmuster aller Areale, wobei die größten Amplituden jeweils vor und hinter dem Hammergriff auftreten, während sie im caudalen Trommelfellbereich etwas geringer sind An menschlichen Felsenbeinpräparaten lassen sich zusätzlich Bewegungen in der Ebene des Trommelfells in den cranialen Teilen der Membran in Richtung auf den kurzen Hammerfortsatz hin nachweisen (Abb 21) Diese Bewegungen sind Ausdruck der großen Bewegungsamplituden in der gewölbten Trommelfellmembran Bei geringen Lautstärken treten sie nicht auf (Tonndorf, pers Mitteilung)

Der distale Teil des Hammergriffes also der Umbobereich wird regelmäßig bei maximaler Auswärtsbewegung des Trommelfells angehoben, während der kurze Hammerfortsatz sich einwärts bewegt In der Mehrheit der Fälle läßt sich auf eine Rotation um das Achsenband der Hammer-Amboß Aufhängung schließen In anderen Fällen wird der kurze Hammerfortsatz um einen größeren Betrag einwärtsbewegt

Bei der Ausbildung von zarten Narben (Abb 19, 2) oder im Experiment in Bereichen, die artefiziell ausgedünnt wurden zeigt sich gegenüber der Norm ein abweichendes Schwingungsverhalten Diese Areale weisen größere Schwingungsamplituden auf als die umgebenden Normalbereiche Bei niederen Frequenzen zeigt sich ein phasengleiches Schwingungsverhalten, während sich mit zunehmender Frequenz Interferenzen ausbilden Als erste Zeichen der Interferenzentstehung ergeben sich wellenförmige Bewegungen also Zeichen dafür, daß die maximalen Schwingungsamplituden zu unterschiedlichen Zeiten erreicht werden Mit zunehmender Frequenz zeigen sich deutlichere Interferenzen, wobei zu gleichen Zeiten Amplitudenmaxima in unterschiedlicher Richtung hervorgerufen werden

Bei der Stroboskopie von eingeheilten Homoio-Trommelfelltransplantaten in vivo lassen sich solche Interferenzmuster beobachten Über ein Wellenstadium kommt es zur Ausbildung ausgeprägter Interferenzen mit 2, 3 oder 4 Amplitudenmaxima zum gleichen Zeitpunkt in unterschiedlicher Richtung In Tierexperimenten an Kaninchen imponiert dieses Verhalten als Schaukelbewegung zwischen z B der vorderen und hinteren Trommelfellhälfte Diese Bewegungsabläufe wurden monocular bei tangentialem Aufblick gefilmt

Nach der Rekonstruktion der Gehörknöchelchenkette durch eine sog Tympanoplastik, bei der durch die Einlage eines Homoio-Amboßes z B auf den Stapeskopf eine säulenförmige Rekonstruktion der Gehörknöchelchenkette vorgenommen wurde, zeigt sich die Trommelfelloberflächenform verändert Sie ähnelt der Membran des Vogelohres mit auswärts vorgewölbtem Endglied der Gehörknöchelchenkette und zeltförmig abfallender Umgebung Daß diese Änderung der Konfiguration keine Minderung der Hörfähigkeit zur Folge haben muß, haben Mes-



Abb. 21 Halbschematische Darstellung der normalen Trommelfellschwingung. Die Pfeile stellen Ortsvektoren dar, schwarz = Auswärts-, weiß = Einwärtsbewegung

sungen am Vogelohr ergeben (122). Auch die große Zahl von Hörverbesserungen, die durch die Tympanoplastiken dieser Form gelingt, bestätigt dies. Auf diese Weise rekonstruierte Trommelfelle weisen analog dem normalen menschlichen Trommelfell ein phasengleiches Schwingungsverhalten im Niederfrequenzbereich und in Abhängigkeit von unterschiedlich dicken Narbenfeldern mit zunehmender Frequenz mehr oder weniger stark ausgeprägte Interferenzerscheinungen auf.

Nach einer reinen Trommelfelloperation, der Myringoplastik, wird [illegible] [illegible] [illegible] einer Perforation implantierte Gewebe in seinen physikalischen Konstanten der normalen Umgebung bei vollständiger Abheilung entspricht. Solche Fälle konnten untersucht werden. Es zeigt sich hier in allen Frequenzen ein phasengleiches Schwingungsbild.

Die Untersuchung der am Trommelfell operierten Kaninchenohren mit unterschiedlich stark ausgeprägten Narbenfeldern führte zu den gleichen Beobachtungen wie nach menschlichen Ohroperationen. Die der Norm entsprechenden Schwingungsvorgänge waren nach Mittelohroperationen am Menschen häufiger zu beobachten als an den Versuchstieren. Dies ist wahrscheinlich durch die größere Operationserfahrung am Menschen bedingt.

G. Diskussion der Ergebnisse

Auf die Frage nach dem normalen Schwingungsverhalten des Trommelfells versuchte als erster Helmholtz (100) eine Antwort zu geben. Eine direkte Messung war ihm noch nicht möglich. Aus einfachen Experimenten mit Volumenänderung im Innenohr und Messung der daraus resultierenden Volumenverschiebung innerhalb des Gehörganges schloß er, daß die eigenartige Konusform des auswärts konvex gebogenen Trichters, den das Trommelfell darstellt, einen wesentlichen Teil des Drucktransformationsmechanismus des Mittelohres darstellt. Die Vorgänge innerhalb der Gehörknochelchenkette wurden ebenfalls von Helmholtz (100) und von Mach und Kessel (157) analysiert.

Im Gegensatz zu den Vorstellungen von Helmholtz, der das Prinzip der gekrümmten Membranen als Drucksteigerungsfaktor in die Physiologie des Mittelohres einführte, fand v. Bekesy (13, 17) einen vollkommen anderen Bewegungsmodus bei direkten Messungen mittels einer kapazitativen Sonde. Aus seinen Meßergebnissen zog er den Schluß, daß das Trommelfell wie ein Türflügel um die Rotationsachse der Gehörknöchelchen, also etwa um das „Achsenband", wie Helmholtz (100) es beschrieben hatte, schwinge. Er fand ein Maximum der Trommelfellbewegung im caudalen Trommelfellanteil, während vor und hinter dem Hammergriff mit zunehmender Annäherung an das Achsenband die Amplituden abnahmen. Die weiteren Untersuchungen auch der Gehörknöchelchenkette und insbesondere der Stapesbewegung und die Entwicklung eines Modells für die Innenohrfunktion von v. Bekesy wurden durch zahlreiche Nachuntersucher bestätigt. Die Angaben von v. Bekésy, daß es sich bei den Schwingungsvorgängen am Ohr in der Nähe der Hörschwelle um unvorstellbar kleine Auslenkungen handeln müsse, führte zu Mißverständnissen (Naftalin (172, 173)).

Die Diskrepanz in den Auffassungen über die Schwingungsform des Trommelfells zwischen den Ansichten von Helmholtz (100) und von v. Békésy (17) wurde durch moderne Untersuchungen unter Verwendung der Laser-Holographie durch Tonndorf und Khanna (134, 135, 136, 226, 227, 228, 229, 230, 231) besonders deutlich. Tonndorf und Khanna fanden die alten Vorstellungen von Helmholtz bestätigt, daß das Trommelfell als wesentlicher Drucktransformator über das Prinzip der gekrümmten Flächen an der gesamten Mittelohrdrucktransformation teilhabe. Sie sahen das Maximum der Trommelfellamplituden vor und hinter dem Hammergriff. Im caudalen Trommelfellanteil konnten sie lediglich geringe Amplituden beobachten.

Unsere Ergebnisse bei stereo-stroboskopischer Betrachtung des Trommelfells unter Verwendung hoher Schalldrücke zeigen, daß das Trommelfell in der von Helmholtz und Tonndorf und Khanna beschriebenen Weise schwingt. Im Gegensatz zu Tonndorf und Khanna mußten wir Schalldrücke zwischen 90 und 120 dB am Normalhörenden verwenden, um diese Schwingungsvorgänge beobachten zu können. Daß Kobrak und Mitarbeiter (140) mit stroboskopischer Untersuchungstechnik eher die Modellvorstellungen von v. Békesy bestätigt fanden, liegt wahrscheinlich daran, daß sie ein monoculares Stroboskop verwendeten. In ihren Beschreibungen findet sich kein Hinweis darauf, daß sie für alle Bereiche des Trommelfells nur Bewegungen vertikal zur Beleuchtungsrichtung beobachteten. Dies ist

bei monocularer Betrachtungsweise unter dem Stroboskop notwendig, da nur solche Bewegungsrichtungen unverkürzt erscheinen

Unter stereoskopischer Betrachtungsweise, wie sie das neu entwickelte Mikrostroboskop gestattet, spielt der unterschiedliche Aufblickwinkel, wie in Voruntersuchungen geklärt werden konnte, keine ausschlaggebende Rolle Bei normalen Trommelfellen fanden wir an menschlichen Felsenbeinpräparaten, an Kaninchen, Ratten und Meerschweinchen und bei der Beobachtung menschlicher Trommelfelle in vivo regelmäßig und in Übereinstimmung mit den Untersuchungen von Tonndorf ein phasengleiches Bewegungsmuster, bei dem die maximalen Amplituden der Einwärts bzw Auswärtsbewegung zu gleichen Zeitpunkten erreicht werden Die Amplitudenmaxima liegen vor und hinter dem Hammergriff, während der caudale Trommelfellanteil eine etwas geringere Auswärts- bzw Einwärtswölbung zeigt

Die Bewegungen der Trommelfelloberfläche bei Beschallung erfolgen vertikal zu Tangenten, die in jedem Punkt radiär an die Oberfläche der gewölbten Trommelfellmembran gelegt werden können Es handelt sich also nicht um eine stempelförmige Ein- oder Auswärtsbewegung sondern um eine komplexe, in jeden Punkt des Trommelfells unterschiedliche Bewegungsrichtung

Zusätzlich zu dieser Ein und Auswärtsbewegung lassen sich mit unserer Beobachtungstechnik tangentiale Bewegungen innerhalb der Trommelfelloberfläche bei Beschallung nachweisen Diese Verziehungen treten insbesondere im cranialen Trommelfellanteil auf und laufen bei Auswärtsbauchung auf den kurzen Hammerfortsatz zu Sie sind umso ausgeprägter, je weiter der Drehpunkt des Hammers zum Umbo hin verlagert wird Wada (239) beobachtete ähnliches bei Vögeln

Diese Verlagerung des Drehpunktes um den der Hammergriff rotiert konnte in zahlreichen Präparaten beobachtet werden Die Beobachtung läßt den Schluß zu daß das „Achsenband" keine starre Rotationsachse darstellt, sondern selbst einwärts und auswärts bewegt werden kann Unsere Befunde bestätigen damit die Ergebnisse von Tonndorf und Khanna (231) u a (71, 87)

Das Bemühen, bei hörverbessernden Operationen ein den natürlichen Verhältnissen entsprechendes Trommelfell zu rekonstruieren, hat im Laufe der Entwicklung der Mikrochirurgie des Ohres dazu geführt, von den anfänglichen Verfahren der Abdeckung z B mit Haut, überzugehen auf Techniken, bei denen Temporalisfascie zum Perforationsverschluß verwendet wird Die postoperativen Resultate wurden auf diese Weise verbessert (96, 186, 210, 244, 252, 253) Gelingt es, eine schlitzförmige Perforation, wie sie nach Knalltraumen in der Regel entsteht, so zu adaptieren, daß die ursprüngliche Faserstruktur bis auf einen schmalen Narbenstreifen erhalten bleibt, so ergeben sich regelmäßig optimale postoperative Ergebnisse Das Schwingungsmuster entspricht dann der Norm

Entsprechende Audiogramm- und Trommelfellbefunde sind in Abb 22 wiedergegeben

Die Faserstruktur des Trommelfells u a von Fumagalli (73), Secondi (208), Lim (147, 148, 149) beschrieben, gewährleistet die optimale Schallaufnahme und Ankoppelung an das Gehörknöchelchensystem Diese Verhältnisse konnte Tonndorf mit der Laser-Holographie an operierten Katzenohren aufklären Aus seinen Analysen folgte die Vermutung, daß bei ungünstigen Ergebnissen gegenphasige Bewegungsmuster im Trommelfell entstehen würden Die direkte Beobachtung dieser gegenphasigen Bewegungsabläufe war mit der Laser Holographie nicht möglich

Unsere Untersuchungstechnik läßt die Beobachtung dieser pathologischen Bewegungsmuster zu Trotz der hohen anzuwendenden Schalldrucke findet sich also nicht nur für den Normalfall sondern auch für pathologisch veränderte Membranen eine Übereinstimmung mit den Ergebnissen von Tonndorf und Mitarbeitern (230)

Im Gegensatz zur Laser-Holographie läßt sich mit dem von uns entwickelten Mikrostro-

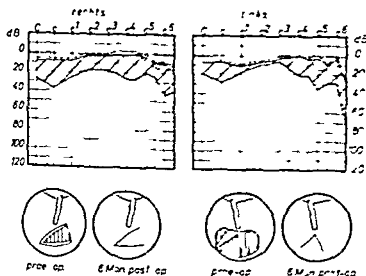


Abb. 22 Audiogramme vor und nach Myringoplastik. Bei einem Knalltrauma zerrissen die Trommelfelle. Die Perforationen ließen sich durch Auskrempeln der Randlappen (Schraffur) fast ganz schließen. Sie heilten unter Bildung zarter Narbenstreifen ab. Schraffur in den Audiogrammen: postoperativer Hörgewinn bis auf die Innenohrleistung

boskop eine Beobachtung des Trommelfells am Menschen in vivo durchführen. Für die Laser-Holographie ist ein Einblickwinkel von mindestens 45° Weite erforderlich, und zusätzlich ist eine absolute Immobilisierung des Objektes notwendig.

Die von Tonndorf theoretisch erarbeitete Forderung nach einer optimalen Ankoppelung zwischen Trommelfell und Gehörknöchelchen wurde durch Plester (186) empirisch für die Myringoplastik entwickelt. Perforationen werden durch Unterlegen mit Temporalisfascie unter die Perforation und unter den Hammergriff und zusätzliches Abdecken mit freien Hauttransplantaten aus der vorderen Gehörgangswand auf die freie Fascie in der Perforation und auf den Hammergriff verschlossen. Auf diese Weise resultiert eine Einscheidung des Hammergriffes und damit eine optimale Ankoppelung.

Durch Verwendung vitaler Temporalisfascie wird ein Material in das Trommelfell eingefügt, das mit seinem hohen Fasergehalt der umgebenden Trommelfellmembran entspricht und so auch nach Abheilung ein analoges Schwingungsverhalten aufweist. Kommt es zu einer Rarefizierung der Kollagenfibrillen in einem Narbenbereich, so treten hier Schwingungsanomalien auf, mit Phasendifferenzen bis zur kompletten Interferenz einzelner Areale. Die wirksame Trommelfellfläche wird durch solche Schwingungsabläufe verkleinert. Es resultiert eine Minderung der Schalldrucktransformation und damit eine Mittelohrschwerhörigkeit.

Daß der Verschluß einer Trommelfellperforation augenblicklich zu einer Hörverbesserung führt, wußte schon Banzer 1640 (6). Yearsley konnte 1848 erstmalig eine Trommelfellperforation mit einer kleinen Wattekugel verschließen, eine Technik, die später Toynbee (zit. n. (138)) propagierte. Der Effekt dieser Maßnahme, die heute als Probeabschirmung regelmäßig bei der Untersuchung von perforierten Trommelfellen in der Klinik angewandt wird, liegt, wie Tonndorf und Mitarb. zeigen konnten (164, 231), in der Minderung des Schalldruckes innerhalb der Pauke. Die Schalldruckdifferenz zwischen medialer und äußerer Trommelfelloberfläche steigt auf diese Weise. Es wird eine größere Auslenkung erreicht.

Die Verwendung von Homoio-Trommelfelltransplantaten nach entsprechender Konservierung geht auf Marquet (162) zurück. Seine günstigen Ergebnisse wurden z. B. von Wehrs (242, 243) auch nach Langzeitbeobachtungen bestätigt. Uns hat sich das Implantieren von Homoio-Trommelfellen bei Totaldefekten reizlosen Mittelohrverhältnissen

währt Die stroboskopische Untersuchung implantierter Trommelfelle weist bei gelungener Einheilung im Mittel- und Niederfrequenzbereich ein der Norm analoges Schwingungsverhalten auf Der Trommelfelltrichter ist etwas flacher geformt als es der Norm entspricht Nur bei hoheren Frequenzen oberhalb von 1000 Hz zeigen sich Interferenzbilder

Im Verhältnis zur Funktionsbestimmung des Trommelfells mit der Impedanzaudiometrie weist die Mikrostroboskopie Vorteile auf Bei der Impedanzmessung wird der vom Trommelfell reflektierte Schall registriert Es handelt sich also um eine über die gesamte Fläche integrierende Meßanordnung, wobei schon im Normalfall die unterschiedlich große, für die Schallübertragung ineffektive Shrapnell'sche Membran mit in das Meßergebnis eingeht Bei operierten Mittelohren wird uber die moglichen Inhomogenitaten des Trommelfells integriert, so daß lediglich pauschale Aussagen über vermehrte oder verminderte Reflexionen von Schall moglich sind (27, 28) Johnstone und Mitarb (123) weisen auf diese Problematik besonders hin Die Paukenimpedanz geht als Unbekannte in die Meßwerte ein

Die Beurteilungsmöglichkeit für eine Myringoplastik nach dem Tonaudiogramm, also die Bestimmung des Schwellenwertes für das Hörvermogen von Sinustönen wird durch zusätzliche Impedanzdaten des Trommelfells kaum verbessert

Anders verhält es sich mit der Impedanzmessung zur Analyse der Funktion der Mittelohrmuskeln Diese laßt sich mit keiner anderen Meßanordnung so genau festlegen wie mit der Impedanzaudiometrie (61 79 178, 179)

Als Untersuchungsverfahren ohne Integration uber die gesamte Flache mit der Moglichkeit, die Einzelareale des operierten Trommelfells zu untersuchen kommen die Laser Holographie oder die Stereo-Mikrostroboskopie in Betracht Wahrend die Laser Holographie eine Labortechnik zur Untersuchung von operierten Versuchstieren ist, gelingt es mit der Mikrostroboskopie, auch menschliche Trommelfelle in vivo zu beobachten Die Ergebnisse beider Verfahren entsprechen sich

Die mikroskopischen Untersuchungen am Menschen in vivo bestatigen die von Tonndorf und Mitarb (231) im Tierexperiment erarbeiteten Ergebnisse, daß die Wiederherstellung der Fasertextur innerhalb der Trommelfellmembran fur das postoperative Schwingungsverhalten von ausschlaggebender Bedeutung ist In unseren tierexperimentellen Versuchsserien konnte weiter gezeigt werden, daß grobe Narbenfelder auch die Schwingungsmöglichkeit des verbliebenen Trommelfellrestes beeintrachtigen konnen Eine sachgerecht ausgefuhrte Myringoplastik resultiert nach vollstandiger Abheilung in einem annahernd normalen Schwingungsverhalten Es zeigen sich dann wie im normalen Trommelfell phasengleiche Schwingungsmuster bis in den hochsten von uns gemessenen Frequenzbereich von 1700 Hz

In Tierexperimenten wurde zusatzlich die Funktion der Gehörknochelchenkette und die Bewegung der runden Fenstermembran beobachtet Diese Untersuchungen bestatigen die aus der Literatur zusammengestellten Auffassungen Es laßt sich bei kontinuierlicher Schalldrucksteigerung beobachten, wie das Amboß Stapes Gelenk bei niedrigen Schalldrucken verzogerungslos Bewegungen des Ambosses uber den Stapes auf das Innenohr ubertragt und bei zunehmender Schallstarke uber eine Subluxation bei der Auswärtsbewegung schließlich durch eine Zerreißung der Gelenkkapsel das Innenohr schützt Die Beobachtung von Interferenzbildern auf der Membran des runden Fensters bei hoheren Frequenzen unabhangig vom Schalldruck laßt den Schluß zu, daß die über den Stapes applizierte Druckwelle innerhalb des Perilymphraumes in Abhangigkeit von der Frequenz auf dem Wege zum runden Fenster Modifikationen erfahrt

H. Zusammenfassung

Die jahrzehntelang akzeptierten Vorstellungen über die Schwingungsform des Trommelfells, wie von v. Békésy (17) sie erarbeitet hatte, konnten Tonndorf und Mitarb. (231) mit modernen Untersuchungstechniken nicht bestätigen. Sie fanden nicht die von v. Békesy postulierte turflügelartige Schwingungsform des Trommelfells um die Rotationsachse der Gehörknöchelchenkette, sondern ein wesentlich komplizierteres Vibrationsmuster. Sie konnten gleichzeitig die mehr als 100 Jahre alten Vorstellungen von Helmholtz (100) bestätigen, daß in der Trommelfellmembran selber ein Hebelmechanismus zu einer Drucksteigerung des aufgenommenen Schalls führt.

Aus diesen kontroversen Versuchsergebnissen und den darauf aufbauenden Theorien resultieren für die Chirurgie des Trommelfells unterschiedliche Operationsprinzipien. Folgt man den Vorstellungen von v. Békesy, so ist bei einer Myringoplastik am Unterrand der Membran ein lockeres Bindegewebe einzubringen, um die notwendigen Freiheitsgrade für die rotierenden Schwingungen des Trommelfells zu erreichen. Entsprechend den Vorstellungen von Tonndorf und Helmholtz wäre gerade in diesem Bereich die Implantation eines festen Bindegewebes analog zu dem übrigen Trommelfell erforderlich, um eine optimale Drucktransformation zu erzielen.

Anliegen unserer Untersuchungen war es, Beobachtungen an menschlichen Trommelfellen in vivo vorzunehmen, um die tatsächlichen Schwingungsvorgänge zu klären.

Nach eingehendem Studium der physiologischen und anatomischen Literatur wurde deshalb ein neues Stereo-Mikrostroboskop entwickelt, das die Beobachtung von Schwingungsvorgängen am Trommelfell auch in vivo zuläßt. Mit dieser Untersuchungsmöglichkeit wurden menschliche Trommelfellpräparate und in Tierversuchsreihen operierte und normale Kaninchen-, Ratten- und Meerschweinchenohren beobachtet.

Nach Selbstversuchen und Beobachtung von normalen Trommelfellen an Menschen in vivo an Freiwilligen, um die Tolerierbarkeit der zu applizierenden hohen Schalldrücke zu prüfen, wurden Patienten nach Ohroperationen untersucht.

Für das normale Trommelfell konnten wir die Ergebnisse von Tonndorf und Mitarb. bestätigen. Die Befunde, die Tonndorf und Khanna für das Schwingungsverhalten operierter Katzenohren erarbeiteten, fanden wir sowohl in den Tierexperimenten als auch nach Ohroperation am Menschen bestätigt. Wir konnten ihre Daten erweitern, da im Gegensatz zu ihrer Untersuchungstechnik unter dem Stereo-Mikrostroboskop Phasendifferenzen und Interferenzbildungen direkt zu beobachten sind. Bei der holographischen Fotographie mit Zeitmittelung entstehen Isoamplitudenlinienbilder in Beleuchtungsrichtung, während unter der Stereomikrostroboskopie eine kontinuierliche Beobachtung aller Bewegungsrichtungen möglich ist. Wir konnten als feinste Anzeichen einer Interferenzbildung regelmäßig Wellenbewegungen innerhalb der Trommelfellmembran beobachten, die mit der Laser-Holographie naturgemäß nicht darstellbar sind. Während bei Laser-Holographie-Technik errechnet werden kann, daß nebeneinanderliegende Amplitudengipfel sich in unterschiedlicher Richtung ausprägen, lassen sich diese Veränderungen als maximale Interferenzbilder unter dem Mikrostroboskop direkt beobachten. Sie lassen sich insbesondere bei stärkeren Vernarbungen oder bei großen, zarten Narbenfeldern regelmäßig im

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Electron Microscopic Assessment of the Cochlea

Some Techniques and Results

BY

IVAN M. HUNTER-DUVAR

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Electron Microscopic Assessment of the Cochlea

Some Techniques and Results

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To Donald N. Elliott
mentor and friend

A Technique for Preparation of Cochlear Specimens for Assessment with the Scanning Electron Microscope

I M Hunter-Duvar

The scanning electron microscope (SEM) provides a surface image, consequently, one of the problems in SEM studies of inner ear structure is the organ's location Solidly embedded in bone the cochlea only becomes accessible to SEM survey after dissection Because of the minute size of the cochlea, dissection must be done under the light microscope and manipulation and distortion of the many fragile cells composing it continues to be a problem The artifacts created during dissection complement, and are sometimes confounded with those occurring during fixation Such preparation artifacts as are tolerable in light microscope studies grow to be of major consequence in the interpretation of SEM micrographs

A decade ago the first SEM studies of inner ear structure were published (Barber & Boyde, 1968, Lim, 1969, Bredberg et al , 1970, Marovitz et al , 1970) The increase in our understanding of inner ear structure that has resulted from SEM studies over the past ten years has been largely a result of researchers' attempts to improve the quality of the specimen that is placed under the electron gun of the SEM (Lim, 1977)

In our laboratory the SEM has proven a valuable tool in studies of small acoustic lesions to the chinchilla cochlea This report describes our efforts to develop a preparation technique that will produce reliable results with cochlear material assessed with the SEM and demonstrates some of the results achieved

METHODS

Animals are decapitated and temporal bones are removed and fixed in 2% phosphate-buffered glutaraldehyde within 10 min of death For fixation, oval and round windows are opened and the fixative is gently perfused through scala tympani and scala vestibuli with a small pipette Cochleas are left in glutaraldehyde for two hours, then rinsed overnight in buffer After a second one hour buffer rinse, specimens are post fixed for 1½ hours in 1% OsO_4 The osmium stains the membranous cochlea so that it can be identified through the bony cochlear capsule, facilitating bone removal and dissection After fixation, temporal bones are rinsed in saline and dehydrated into 70% alcohol for dissection

Our acoustic trauma studies deal with lesions resulting from exposure to a 1 kHz pure tone When lesions from the stimulus occur they are centered approximately 9 mm from the basal end of the cochlea (Hunter-Duvar and Bredberg, 1974) The lower middle and upper basal turns are therefore of greatest interest to us and our efforts are directed at the best possible preservation of structure in that area

Dissection consists of thinning the bony cochlear capsule with a diamond dental burr until the osmium darkened spiral ligament is clearly visible through the bone (Engstrom et al , 1966) Beginning at the apex the bone is removed from around the membranous cochlea until the lesion area is reac

is taken to leave sufficient bone to support the spiral ligament in the turn containing the suspected lesion area. The apical turn and the upper middle turn are gently removed with iris scissors. This leaves one half turn exposed above the lesion area and one half of a turn exposed below it. Reissner's membrane is still in place over the lesion area at this time, however, with the light microscope, the area is clearly visible through both Reissner's membrane and the tectorial membrane. Reissner's membrane is removed next and the stria vascularis and spiral ligament are picked away from the supporting bone which is left at a level slightly above that of the basilar membrane. The tectorial membrane remains in place on the organ of Corti. Since the spiral ligament is still attached to the bony cochlear wall this procedure results in the least possible manipulation of the organ of Corti and its supporting structures.

After dissection, specimens are prepared for SEM examination without an evaporated metal coating (Malick & Wilson, 1975). This osmium-thiocarbohydrazide (OTO) preparation consists of running specimens back into distilled water then immersing them in a saturated filtered solution of thiocarbohydrazide (TCH) for 20 minutes. This is followed by six rinses in distilled water and 2 hours incubation in 1% OsO_4. The above steps are repeated and the specimens are then dehydrated through 100% alcohol or acetone, dried in a critical point dryer, and mounted on stubs with colloidal silver as an adhesive for viewing with the SEM.

RESULTS & DISCUSSION

Critical point drying of biological specimens results in varying degrees of shrinkage depending on the material. We have measured cochlear shrinkage as approximately 21% of the total membranous cochlea area by using the bony cochlear wall as a reference (Hunter-Duvar, 1977). The osmium-thiocarbohydrazide (OTO) technique tends to confine the shrinkage artifact in the organ of Corti to large specific cracks (Fig. 1) leaving cell walls and junctions generally well preserved. This aspect of the shrinkage is advantageous in that the cracks formed are often wide enough to allow assessment of the hair cell bodies, pillar cells, and tunnel crossing fibers in areas where cracks occur (Fig. 2). The nature of the shrinkage pattern causes the tectorial membrane to turn up at the point of its attachment to the limbus (Fig. 1) and both the upper surface (Fig. 3) and the underside (Fig. 4) of the membrane may be examined by tilting the specimen. Figure 4 also demonstrates the variation in attachment of Hensen's stripe to the membrane in lower and upper turns. Since the position of the tectorial membrane relative to its point of contact with cilia of outer hair cells remains fixed, possible alterations to that relationship from treatment can be determined (Fig. 5).

Preservation of cell surfaces and boundaries as demonstrated in Fig. 6 is superior to all other preparation methods we have attempted. Preservation of stereocilia is excellent (Fig. 7). The relative density of microvilli on cell surfaces bounding the scala media is clearly shown and we note the similarity in surface structure between Claudius cells (Fig. 8) and those of the underside of the endolymphatic layer of Reissner's membrane (Fig. 9). Alterations in the pattern of microvilli populations such as occur in ototoxic drug damage can be assessed (Fig. 10). A comparison of Fig. 8 and 9 with Fig. 11 and 12 demonstrates the differences in the surface structure of cells bounding endolymphatic space (8, 9) and those bounding perilymphatic space (11, 12). The kinocilia on tympanic layer cells previously reported by Angelborg & Engstrom (1974) are well demonstrated (Fig. 12). Additionally shown are the differences in the density and structure of basilar membrane fibers in middle (Fig. 13*a*) and apical (Fig. 13*b*) turns of the cochlea.

The OTO technique has another advantage in that the specimen may be fractured and

scanned without the problem of electron charging This feature has allowed us to look at, among other things the interior of cells (Fig 14) and of the tectorial membrane (Fig 15), as well as the innervation patterns of outer hair cells (Fig 16)

Throughout our experiments acoustically damaged cochleas have presented special problems with SEM assessment Damaged structures appear to become much more fragile and subject to cracking and destruction during preparation Although this problem does not disappear with OTO prepared specimens (Fig 17), it is considerably alleviated, and assessment of the reticular surface (Figs 18, 19) stereocilia (Fig 20) and tunnel structures (Fig 21) immediately after exposure is facilitated The preparation procedure has proven reliable enough to allow us to assess the effects on stereocilia of pure tone exposures that result in only temporary hearing losses By rotating specimens in the SEM and photographing them we are able to obtain composites showing in detail the condition of the stereocilia over a full turn of cochlea Figure 22(*b*) demonstrates only ½ mm of organ of Corti in a composite that contains 5 mm of the organ of Corti

Material prepared for SEM by this method may be rehydrated, embedded in plastic and thin sectioned There is of course no metal coating to section through Some loss of detail is apparent (Fig 23) but material is suitable for survey purposes One disadvantage encountered is that the blackened state of the specimens makes difficult the location of specific areas for orientation

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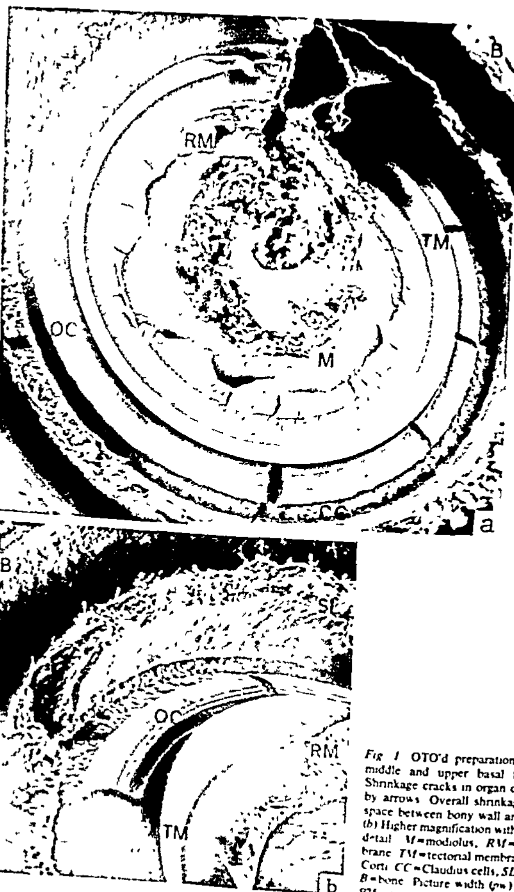


Fig. 1 OTO'd preparation containing lower middle and upper basal turns of cochlea. Shrinkage cracks in organ of Corti are shown by arrows. Overall shrinkage is indicated by space between bony wall and spiral ligament. (*b*) Higher magnification with greater structural detail. *M* = modiolus, *RM* = Reissner's membrane, *TM* = tectorial membrane, *OC* = organ of Corti, *CC* = Claudius cells, *SL* = spiral ligament, *B* = bone. Picture width (*pw*) *a* = 2300 μm, *b* = 925 μm

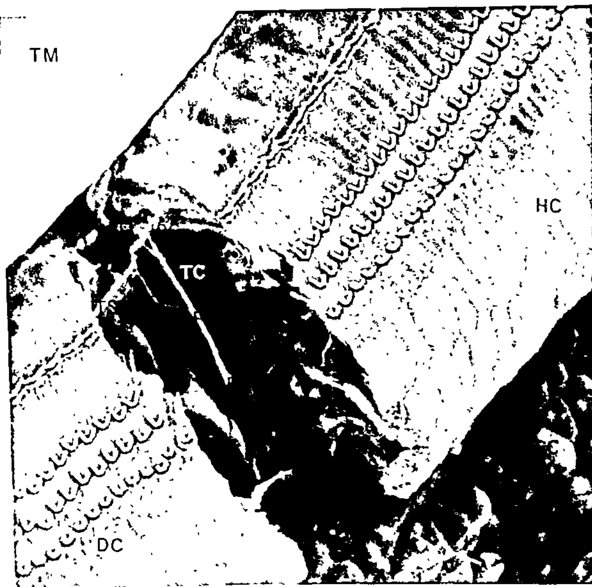


Fig 2 ... internal structure of organ of Corti. *TM*=tectorial membrane, *I* ... radial fibers, *IPC*=inner pillar cell, *OP* ... =Hensen's cells, *CC*=Claudius cells, *TC*

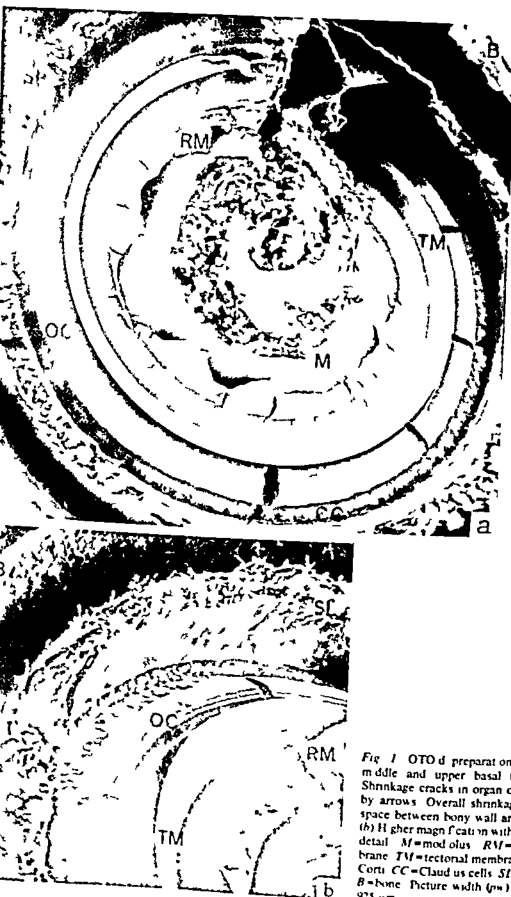


Fig 1 OTO d preparat on contain ng lower m ddle and upper basal turns of cochlea. Shrinkage cracks in organ of Corti are shown by arrows Overall shrinkage is ind ated by space between bony wall and sp ral ligament (*b*) H gher magn f cati n with greater structural detail *M* = mod olus *RM* = Reissner's membrane *TM* = tectonal membrane *OC* = organ of Corti *CC* = Claud us cells *SL* = sp ral l gament *B* = bone Picture width (pw) *a* = 2 300 µm *b* = 975 µm

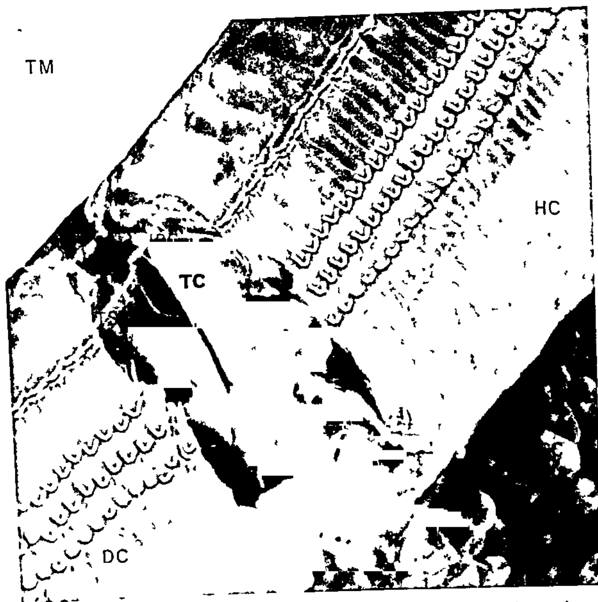


Fig 2 St ... brane *HI* ... cell *OPC* ... cells *TC*=

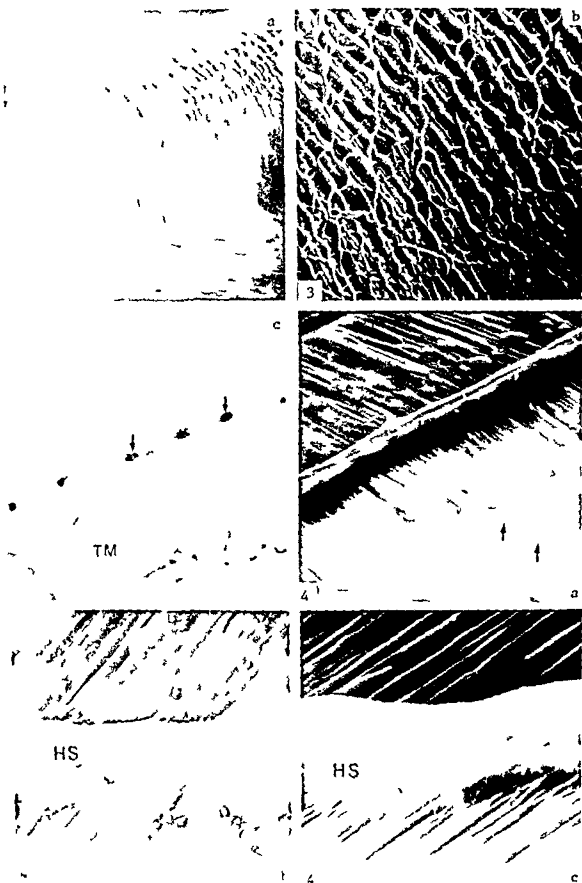
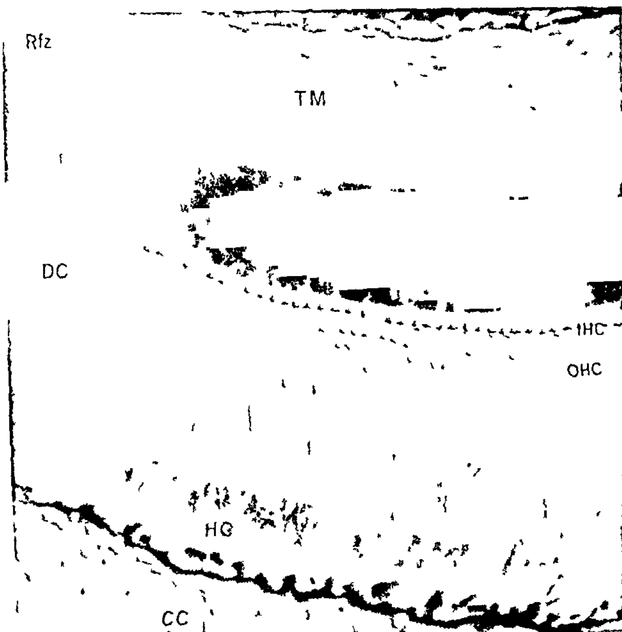


Fig. 3 Lower surface of tectorial membrane [illegible] surface fibers in greater detail. OHC = outer hair cell [illegible] nal mem[illegible] [illegible] μm [illegible] μm [illegible] μm

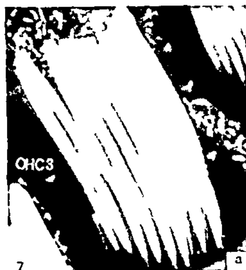


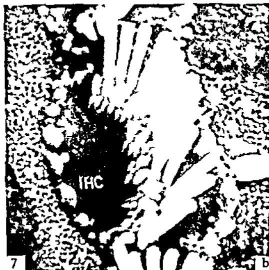
F g 5 At h gher magn f cat on imprints of ind vidual c l a can be def ned and imprints in underside o' tectonal membrane in (*a*) come from c lia on hair cells shown in (*b*) pw *a*=25 μm *b* ?0 μm

F g 4 Unders de of tectonal membrane showing imprints from cilia of outer hair cells (*arrows*) and Hensen s stripe (*HS*) Difference in the attachment of Hensen s stripe to tectonal membrane in (*b*) lower m ddle turn and (*c*) ap cal turn is demonstrated pw *a* 50 μm *b*=5 μm *c* 5 μm



F *c* *6* Area f normal rgan f Cort dem nstrat ng qual ty of spec mens prepared by the OTO meth d *TM* = tect rial membrane *Rf* = Randfa ernetz *HS* = Hensen s stripe *ISC* = nner sul us cells *IHC* = ha of inner hair cells *OHC* = l a of outer ha r cells *DC* = ret ular plates of De ters cells *HC* = Hensen s cells *CC* = Claud us cells pw = 100 μm







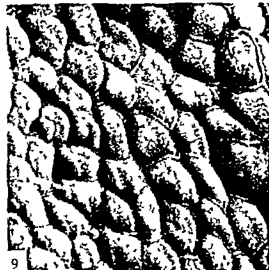


Fig 7 Cilia on hair cells (*a*) third row outer hair cell (*OHC3*) lower apical turn (*b*) inner hair cell (*IHC*) middle turn pw $a=5\ \mu m$ $b=9\ \mu m$

Fig 8 Endolymphatic surface of Claudius cells *HC*=Hensen cells pw=80 μm

Fig 9 Endolymphatic surface of cells of Reissner s membrane pw=40 μm

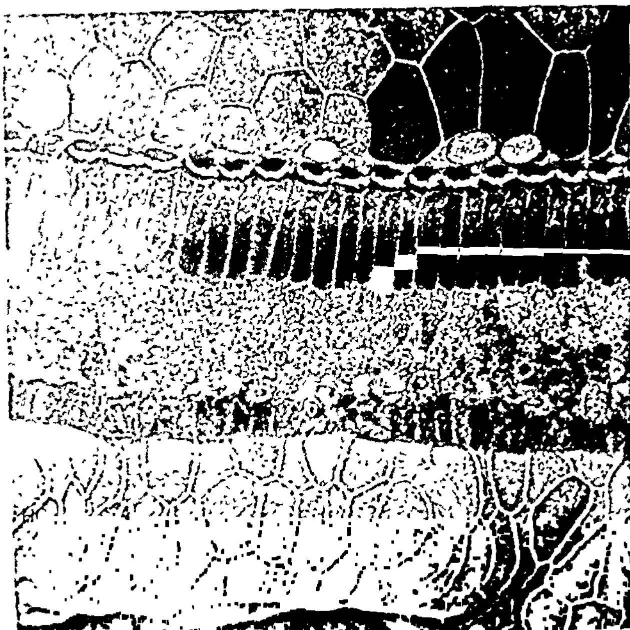


Fig. 10. Specimen from middle turn of an animal treated with an ototoxic antibiotic. Note the tremendous overgrowth of microvilli (*m*) in the area once occupied by the outer hair cells. *IHC* = inner hair cells. *PC* = reticular plates of inner pillar cells. *DC* = reticular plates of Deiters cells. *HC* = Hensen cells. pw = 130 μm

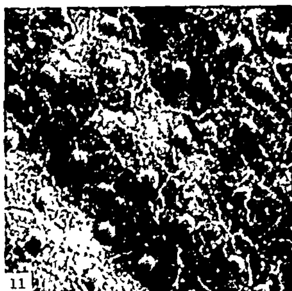






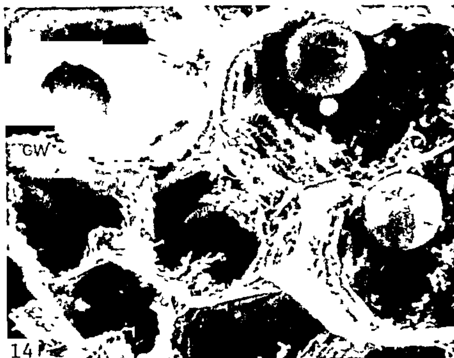
Fig 11 Mesothelial cells of the perilymphatic surface of Reissner's membrane Bulges contain nuclei of the thin cells pw=150 μm

Fig 12 Mesothelial cells of the tympanic layer on the perilymphatic side of the basilar membrane in the middle turn (*b*) Kinocilium (K) on tympanic layer cell pw *a*=70 μm *b*=5 μm





Fig. 13. Fibers (*F*) of the basilar membrane seen through cells of the tympanic layer (*TLC*). (a) closely packed fiber bundles of the lower middle turn are contrasted with the well separated fiber bundles of the apical turn (b). a = 8 μm, b = 45 μm



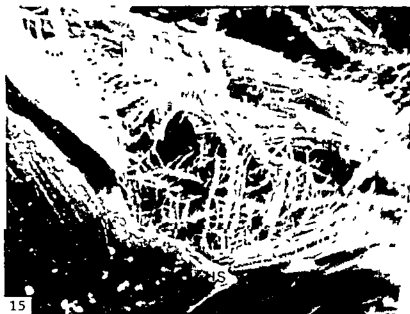
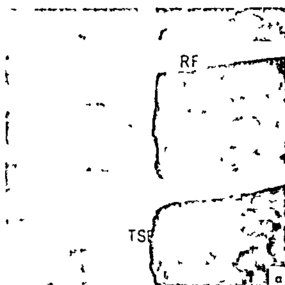
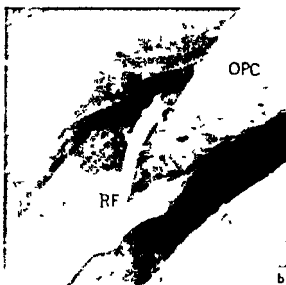


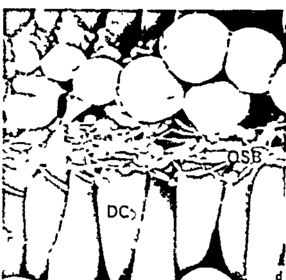
Fig 14 Top view of cells of the tympanic layer showing nuclei (N) [illegible]
pw=120 μm

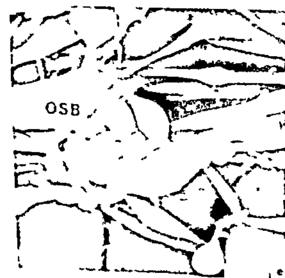
Fig 15 Fracture through tectorial membrane showing nature of [illegible]
(*F*) *HS*=Hensen s stripe pw=35 μm











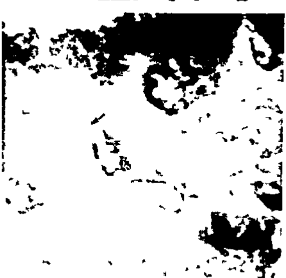


Fig. 18. Nerve fibers of the tunnel of Corti, space of Nuel and habenula perforata. (a) Tunnel spiral bundle (TSB) with radial tunnel crossing fibers (RF) branching off. Note the relative sizes. (b) Radial

[illegible]

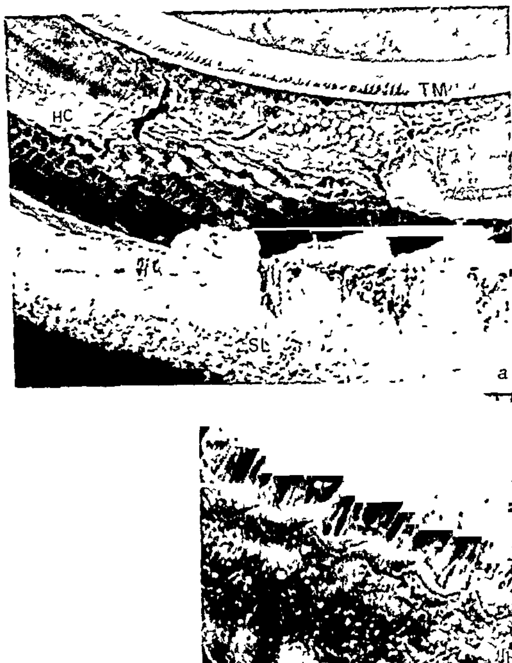


Fig 17 Acoustic pure tone lesion 9 months post-exposure Shrinkage cracks occurring in area are shown by arrows Inner sulcus cells (*ISC*) Hensen cells (*HC*) and Claud us cells (*CC*) have over grown the lesion area and sealed the tunnel of Corti (*b*) Tectorial membrane (*TM*) from over lesion shown in (*a*) still shows cilia imprints (*arrows*) 9 months after organ of Corti destruction *SL*=spiral l gament pw *a* 600 μm *b*=32 μm

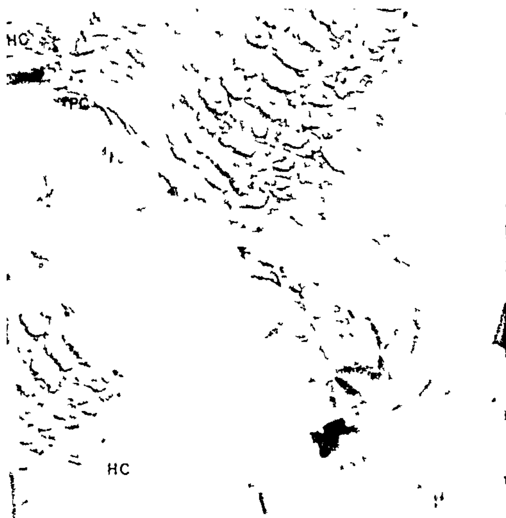
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fibers passing between outer pillar cells (*OPC*) (*c*) Radial f bers and their connections to the outer spiral bundle (*OSB*) (*d*) The f rst outer spiral bundle (*OSB*) and its relation to Deiters cells (*DC*) and the [illegible]

[illegible] e 8 μm f=15 μm



F g 18 S ...
(*OHC 1*) and are fused and are undergoing autolysis on the second row outer hair cells (*OHC2*). Cilia on OHC3 are fused on some cells but no autolysis is evident. pw 55 μm



21 Shrinkage crack through organ of Corti in les on area mal 5 min post
posure A comparison w th Fig 2 shows a ה of o ls and sup-
rting De ters cells has resulted from the sound r p llar ,inner pillar
1 *HC*=Hensen cells *CC*=Claud us cells *IHC* 50 μm

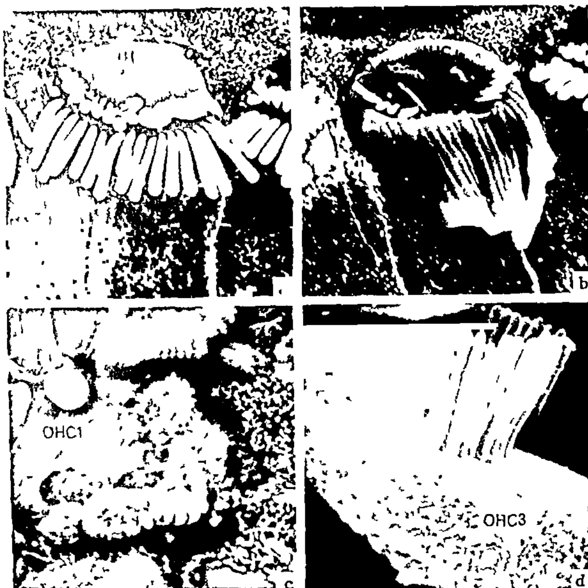
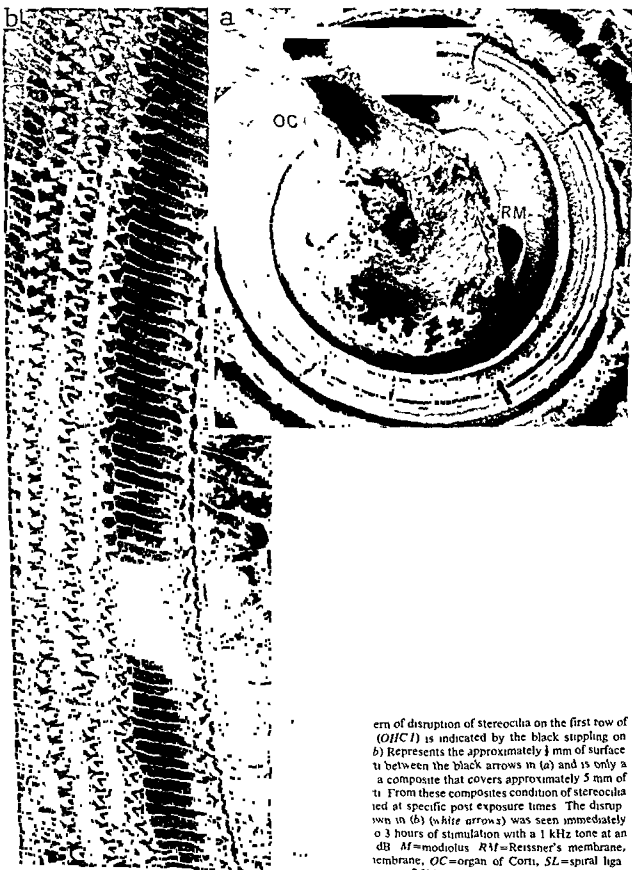


Fig 20 Damaged stereocilia can be read ly resolved Damage d ffers greatly depending on the loca tion and severity of trauma (*a*) Inner hair cell with cilia bent over (*b*) Inner Hair cell with cilia bent over and fused (*c*) OHC 1 cilia in state of autolysis immediately after sound exposure (*d*) OHC3 cil a still intact on cuticular plate which has been ejected from organ of Corti 24 hours after exposure pw *a*=9 μm *b*=9 μm *c*–6 μm *d* 3 μm


HC



ern of disruption of stereocilia on the first row of (*OHC1*) is indicated by the black stippling on *b*) Represents the approximately ⅓ mm of surface ti between the black arrows in (*a*) and is only a a composite that covers approximately 5 mm of ti From these composites condition of stereocilia ied at specific post exposure times The disrup own in (*b*) (*white arrows*) was seen immediately o 3 hours of stimulation with a 1 kHz tone at an dB *M*=modiolus *RM*=Reissner's membrane, iembrane, *OC*=organ of Corti, *SL*=spiral liga pw a=2500 μm, b=350 μm

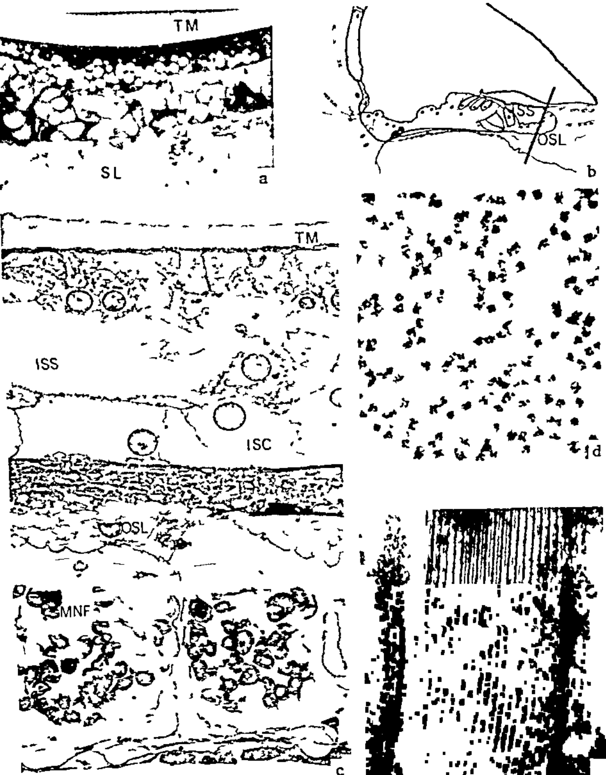


Fig 23 (*a*) Scanning electron micrograph showing the pure tone lesion in the cochlea of an animal 9 weeks post-exposure The specimen was OTO d prior to critical point drying and scanning After rehydration and embedding the area containing the lesion was thin sectioned and assessed with the transmiss on electron microscope (*b*) Line shows location and orientation of sections shown (*c*) Unstained micrograph shows myelinated fibers remaining in osseous spiral lamina in area where organ of Corti has been completely destroyed (*d*) Higher magn fication showing fibers of tectorial membrane (*e*) Myelin sheath on nerve fibers *TM*=tectorial membrane *L*–limbus *ISS*=inner sulcus *ISC*=inner sulcus cells *MNF*=myelinated nerve fibers *OSL*=osseous spiral ligament *LS*–spiral lamina pw a=300 μm c=60 μm d=0 6 μm e=0 2

Reissner's Membrane and Endocytosis of Cell Debris

I M Hunter Duvar

Structure and ultrastructure of Reissner's membrane (Reissner 1851) have been described by Duvall & Rhodes (1967) and Johnsson (1971, 1973) among others The function of this bi cellular wall which separates scala vestibuli from scala media remains obscure Several studies (Ilberg, 1968, Hinojosa, 1971 Duvall & Quick, 1969, Angelborg, 1974) have used various tracers to determine the permeability of Reissner's membrane From the results of these studies it would appear that, although both the scala vestibuli cell layer and the scala media cell layer pick up tracer particles transport through the membrane only occurs from scala vestibuli to scala media

During the course of a recent chinchilla study concerning the effects of pure tone stimulation on cochlear ultrastructure, unusual deposits were seen in one of the animals on Reissner s membrane immediately above the lesion to the organ of Corti Subsequent investigation demonstrated that these deposits consisted of debris from the organ of Corti lesion Electron microscopy suggested endocytosis of cochlear debris by Reissner's membrane Investigations of specimens of Reissner s membrane from other animals gave results which supported the hypothesis

MATERIALS AND METHODS

Initially, debris was found on Reissner's membrane of a chinchilla exposed to a 1 kHz pure tone stimulus at an intensity of 120 dB for 7 minutes and decapitated 10 weeks after exposure The cochlea was immediately fixed by perfusion through round and oval windows with 2 5% phosphate buffered glutaraldehyde, post fixed with 1% osmium tetroxide, and dehydrated to 70% alcohol for dissection The bony cochlear wall was thinned with diamond dental burrs and the bone removed to one half turn above the pure tone lesion, which was clearly visible under the dissecting micro scope The top turn of the cochlea was removed and the lesion area was photographed through the intact Reissner's membrane The portion of Reissner's membrane containing the debris was removed with iris scissors and photographed while in 70% alcohol The Reissner's membrane specimen was dehydrated, embedded and then sectioned for evaluation with the transmission electron microscope The cochlea containing the organ of Corti lesion area was OTO'd (Hunter-Duvar, 1977) and critical point dried for subsequent evaluation with the scanning electron microscope

Specimens of Reissner's membrane from cochleas of unexposed chinchillas as well as from sound exposed and ototoxic drug treated animals were processed in a similar manner In some cases Reissner's membrane was not removed prior to cochleas being critical point dried so that it could be assessed with the scanning electron microscope

RESULTS

Figure 1 is a low magnification light micro graph with focus slightly above the organ of Corti lesion and shows the foreign material attached to the endolymphatic side of Reiss ner s membrane The distribution of patches of debris on the scala media side of a speci men of Reissner's membrane which has been dissected away from over the lesion area, is shown at higher magnification in the left inset

(*a*) and the nature of the organ of Corti lesion is shown in the scanning micrograph in the right inset (*b*) Debris was also located with the scanning electron microscope on Reissner's membrane in other sound exposed and drug treated animals (Fig 2) The debris was localized in small patches Some of the patches were bounded with a membrane (Fig 3) while others appeared to just hang together (Fig 4) Vesicular invaginations and coated vesicles were present in large numbers in the cell walls of both sides of the epithelial cells of the endolymphatic cell layer (ECL) (Figs 5, 7, 8) These invaginations and vesicles were also present in smaller numbers on the underside of the mesothelial cells of vestibular cell layer (VCL) (Figs 7, 4, 8) and occasionally seen on the scala vestibuli or upper side of the VCL (Fig 5) Lysosomes were present in large numbers in the ECL (Figs 5, 6, 7) Lysosomes, occasionally seen in the thin layer of the VCL (Fig 7), were numerous in the swellings surrounding the nuclei of the VCL (Figs 7, 8) These nuclear areas of the VCL showed signs of considerable activity as judged by the quantity of organelles and vesicles (Fig 7, 8) Golgi apparati were regularly observed in the ECL (Fig 4, 7), but infrequently in the VCL

At several debris locations, cells of the ECL appeared to have altered their normally flat shape and expanded their surface area with large protrusions into or around the debris (Figs 3, 6) In several areas micrographs gave the impression of an attempt by the cells to engulf entire patches of debris with these protrustions (Fig 6 inset) Micrographs did not always demonstrate absorption of debris by a process of vesiculation as in some areas no cell wall appeared to exist between debris and cytoplasm (Fig 9) Serial section ing and specimen tilting failed to solve the enigma of the missing membrane in such areas

The perilymphatic surface of the VCL was dotted with unidentified electron dense particles approximately 0 15 to 0 25 μm in size They were only found on the perilymphatic surface The VCL contains many pores (Figs 6, 10) and in the areas of these pores the particles were noted to penetrate the VCL to the basement membrane They seemed related to the perilymphatic cell surface of the VCL (Figs 4, 7 inset) The particles were found on occasion in specimens from cochleas of unexposed animals prepared in the same way

DISCUSSION

Duvall & Quick (1969) noted large numbers of lysosomes in scala media cells of Reissner's membrane and in Claudius cells of animals with cochlear damage and postulated ingestion of cochlear debris by Reissner's membrane Our results appear to confirm that hypothesis, as the adhesion of the debris and the vesicular and lysosomal activity of the ECL are strong evidence for ingestion or endocytosis Altera tions seen in the endolymphatic surface of the ECL also suggest transport from scala media into the cells Wustrow (1952) and Johnsson (1971) have suggested protrusions seen on the ECL are cells being shed into the scala media by the epithelial cells Unfortunately micrographs depicting the phenomenon are not clear enough to determine if theirs is the same process as that described above

Duvall & Quick did not find lysosomal activity in the VCL of Reissner's membrane from their animals with acoustic trauma damage and concluded therefore that "debris within scala media never passed into scala vestibuli" The above findings indicate that although lysosomes are not numerous in the thin flat cellular portions of the mesothelial cells of the VCL, they are present However, in the cytoplasm surrounding the nucleus of the VCL cells, vesicular and lysosomal activity was at least equivalent to that seen in the ECL Hinojosa's (1971) study of ferritin transport across Reiss ner's membrane would argue against a rela tionship between the debris and the activity in the VCL Since directionality of endocytosis cannot be determined from our data, origin

of lysosomes and activity in VCL, nuclear areas must remain a question at this time

The electron dense particles on the perilymphatic surface of the VCL are another interesting phenomenon The particles appear to have a definite relationship to the VCL It is tempting to view them as residual-body products of exocytosis from the cell debris that have been eliminated by defecation from the VCL Such a speculation would of course run counter to the findings of Hinojosa and other explanations must be given consideration The particles may be a product of the perilymphatic space that are static and simply resting on the membrane Alternately they may be a product of the perilymphatic space being transported through Reissner's membrane accounting for the vesicular and lysosomal activity in the VCL Further experiments are necessary before a choice may be made among these alternatives

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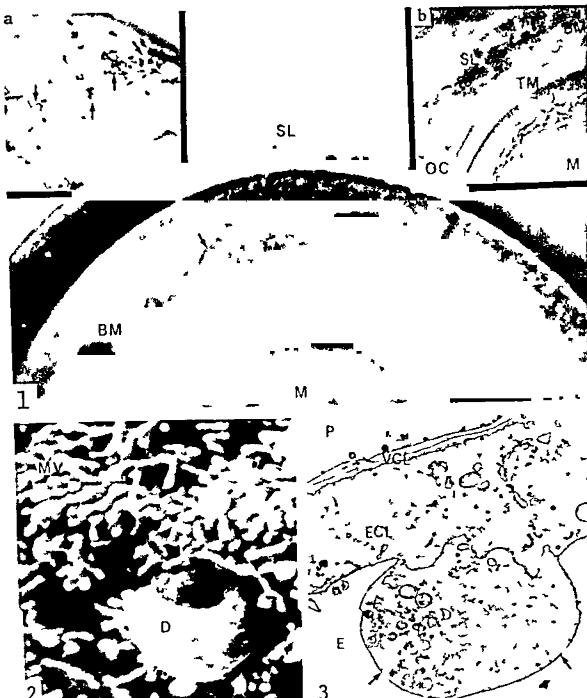


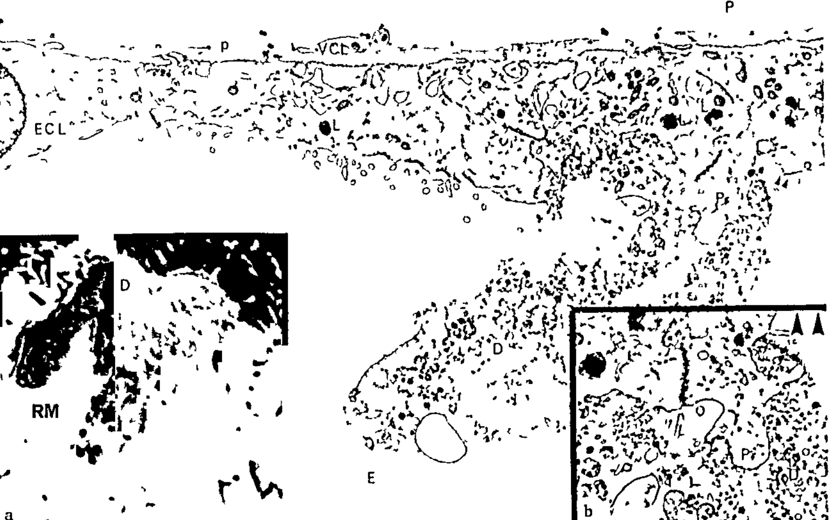
Fig 1 Light micrograph showing debris (*arrows*) on Reissner's membrane over lesion area 10 weeks post-exposure Inset (*a*) shows Reissner's membrane (*RM*) with debris (*arrows*) at a higher magnification Inset (*b*) is a scanning electron micrograph showing the nature of the lesion *SL* spiral ligament *OC* organ of Corti *BM* basilar membrane *TM* tectorial membrane *M* modiolus Picture width (pw) 2 mm $a=750\ \mu m$ $b=750\ \mu m$

Fig 2 Scanning electron micrograph showing membrane bounded debris (*D*) on endothelial cells of endolymphatic side of Reissner's membrane (*RM*) *MV* microvilli pw 4 µm

Fig 3 Micrograph demonstrates a membrane (*arrows*) bounding debris (*D*) attached to e lymphatic side of Reissner's membrane Protrusions are seen to greatly expand the cell surfa at locations of debris *VCL* vestibular cell layer *E*=endolymphatic space *P* perilympha *ECL* endolymphatic cell layer pw=4 8 µ

Fig 4 Debris (*D*) with no bounding membrane on endolymphatic side of Reissner's membrane *VCL*=vestibular cell layer *P*=perilymphatic space *ECL*=endolymphatic cell layer *E*=endolymphatic space *N*=nucleus *G*=Golgi apparatus *L*=lysosome pw=18 8 μm

Fig 5 Large numbers of invaginations (*arrows*) are present on both surfaces of the endothelial cell A single invagination is shown on the vestibular side of the mesothelial cell *VCL*=vestibular cell layer *ECL*=endolymphatic cell layer *L*=lysosome *E*=endolymphatic space *P*=perilymphatic space pw=2 μm



g 6 Numerous lysosomes (*L*) are evident in debris (*D*) areas Endothelial of the endolymphatic cell layer (*ECL*) are seen to protrude into the debris give an appearance of attempting to enclose portions of it Electron-dense erial (*arrows*) on the vestibular s de of the vest bular cell layer (*VCL*) is seen to penetrate to the basement membrane at pores (*p*) Inset (*a*) is a scann ng micrograph of debris that is not bounded by a membrane Inset (*b*) shows protrus ons (*Pr*) of endothel al cells at higher magn ficat on pw=24 μm a=5 μm b=4 4 μm

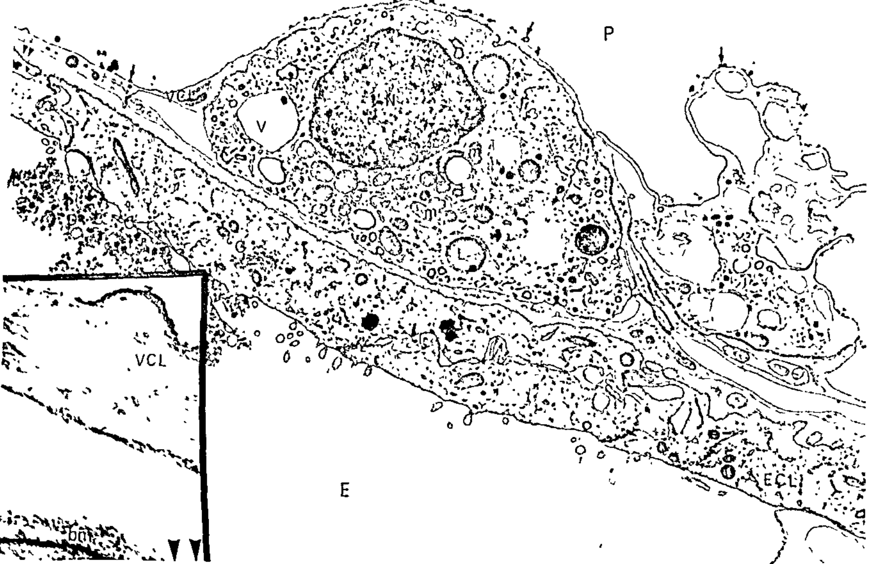


Fig. 7 Area of mesothelial cell of the vestibular cell layer (*VCL*) containing a nucleus Numerous organelles are seen here in both cell layers of Reissner's membrane indicating high activity in an area where cell debris (*d*) is present in the endolymphatic space (*E*). Electron dense particles (*arrows*) are seen in the perilymphatic space of scala vestibuli on the vestibular surface of the mesothelial cells As indicated in the inset these particles appear to be associated with the upper surface of the mesothelial cell. *N*=nucleus, *V*=vacuole, *G*= Golgi apparatus, *L*=lysosome, *m*=mitochondrion, *bm*=basement membrane. pw=20 μm, inset=0 4 μm.

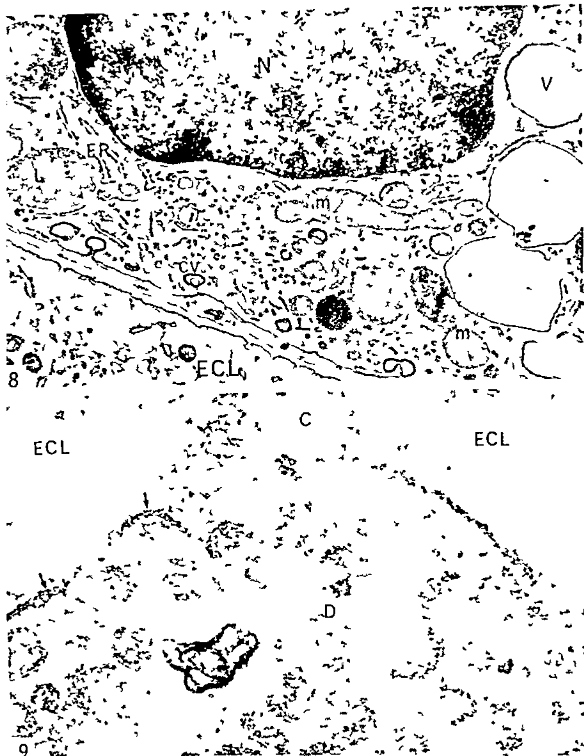


Fig 8 M crograph demonstrates tremendous organelle activity in nuclear area of mesothel al cell in vicin ty of debris *ECL* endolymphatic cell layer *N* nucleus *L* lysosome *V* vacuole *m*—m tochondrion *CV* coated vesicle *ER* endoplasmic reticulum pw 6 μm

Fig 9 Is typical of areas where cell membrane (*arrows*) could not be found between the cytoplasm (*C*) of the endothel al cells and the debris (*D*) in the endolymphat c space pw=0 9 μm

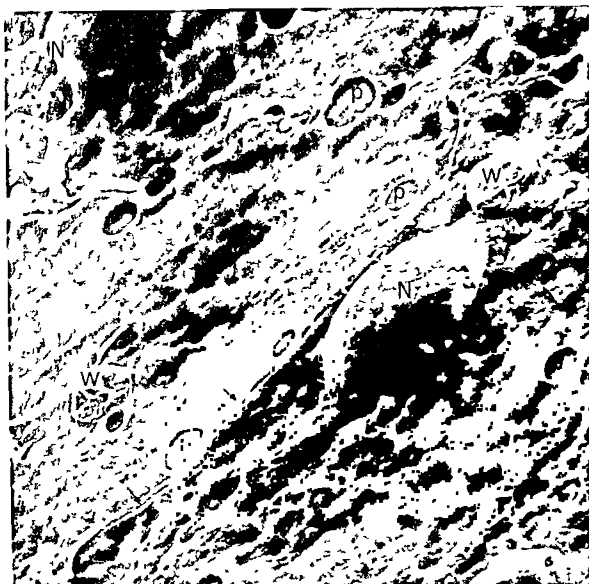


Fig 10 Scanning electron micrograph of mesothelial cells on upper surface of Reissner's membrane Pores (*p*) through cell layer are clearly shown as are bulges (*N*) containing cell nuclei Loosely joined cell borders (*arrows*) are well defined The smaller bulges (*W*) on the cell surface probably represent the whorls of mesothelial cells shown next to the nuclear area in Fig 7 pw = 170 μm

A Technique for Preparation of Large Cochlear Specimens for Assessment with the Transmission Electron Microscope

I M Hunter-Duvar and R J Mount

Our current studies attempt to correlate small behaviorally determined permanent hearing losses with initial changes in inner ear anatomy Pre exposure thresholds are obtained from avoidance conditioned chinchillas A complete description of training, testing and sound exposure procedures is available in Hunter-Duvar & Bredberg (1974) Animals are exposed to pure tone stimulation of sufficient intensity and duration to produce a permanent hearing loss of 10 dB to 20 dB Permanent hearing losses are determined from post-exposure threshold levels approximately 10 weeks after exposure

Histological techniques previously used (Bredberg & Hunter Duvar, 1975) allowed us to inspect the overall condition of hair cells of the organ of Corti with the light microscope and then select areas of interest for routine inspection of ultrastructure with the transmission electron microscope (TEM) On occasion we found animals which exhibited permanent hearing losses but did not show hair cell damage that was visible with the light microscope A technique was needed that would allow us to survey large areas of cochlea at low power with the TEM, while retaining the capacity to inspect ultrastructure at high magnification This paper describes the procedure developed for that purpose

METHODS & RESULTS

The material shown comes from normal chinchillas and chinchillas exposed to a 1 kHz pure tone stimulus of sufficient intensity and duration to result in cochlear lesions of varying degree Lesions from the 1 kHz tone occur approximately 9 mm from the basal tip of the cochlea (Hunter-Duvar & Bredberg 1974)

Animals are decapitated and temporal bones are immediately removed and fixed within 10 min of death Initial fixation is by perfusion of 2 5% phosphate buffered glutaraldehyde through scala vestibuli and scala tympani from oval window to round window after removal of the stapes and the round window membrane Temporal bones are then immersed in the fixative for 2 hours and rinsed overnight in buffer They are post fixed the next day for 1 to 2 hours in 1% phosphate-buffered osmium tetroxide The temporal bone is rinsed in saline and run through graded alcohol rinses into 70% alcohol for dissection (Engstrom et al , 1966)

The bony cochlear wall is thinned with a diamond dental burr until the osmium darkened membranous cochlea of all turns is clearly visible through it The thinned bone is dissected away from over apical, middle, and upper basal turns, with fine forceps The thin layer of bone covering the spiral ligament in lower middle and upper basal turns is not removed The apical turn and the upper half of the middle turn are removed with fine iris scissors Reissner s membrane is left intact over lower middle and upper basal turns This area is closely inspected with the dissecting microscope for any sign of lesions to the organ of Corti or supporting cells When such lesions

are evident they are recorded by photo graphing them through the intact Reissner's membrane (Fig 1) The temporal bone and the removed turns are then dehydrated and embedded in Epon or Spurr

Plastic and bone are removed from around the lower middle and upper basal turns and this area is cut away with sharp razor blades (Bohne, 1972, Spoendlin & Brun, 1974) Excess plastic is trimmed away and specimens are re embedded in plastic in a mold made from a standard 11 mm×75 mm×1 mm glass slide Specimens embedded in the plastic slides are examined under the phase micro scope This procedure allows complete inspection of the organ of Corti and surrounding structures at the upper limits of resolution of the light microscope prior to the preparation of selected areas for the TEM (Fig 2) Resolution achieved with specimens em bedded in the plastic slides is comparable to that obtained with glycerol slide preparations or to specimens viewed in alcohol under the inverted microscope

Areas of interest of approximately 1 mm in length are cut out of the plastic slides and re embedded in flat molds to allow desired orientation Specimens are mounted for cutting with an orientation that allows sectioning the specimen beginning at the lateral boundary of the spiral ligament perpendicular to the basilar membrane (Fig 3) The curvature of the cochlea makes it impossible to section through the same area all along the knife blade on long sections Consequently when both ends of a section are showing the Hensen cells the center area is showing the second row of outer hair cells

The specimen is thick and thin sectioned from spiral ligament through stria vascularis Claudius cells and Boettcher cells (Fig 4) Hensen cells (Fig 5) and inner hair cells (Fig 6), to the inner sulcus cells Sections are cut 0 5 to 1 0 mm in length It is of course not possible to reproduce a full millimeter of cochlea on a journal page format and maintain any detail For that reason the micrograph composites shown herein cover only a portion of the actual length of specimens from which they were obtained The higher power micrographs accompanying the composites give some idea of the detailed information that is available from the sections

Orientation is dependent on information desired The specimen holder can be adjusted to allow sections to be cut parallel to the cell bodies of the outer hair cells (Fig 7) When the tunnel of Corti is reached the angle of the specimen holder may be changed approximately 60° so that the angle of cutting is parallel to the bodies of the inner hair cells (Fig 8) At this angle when inner hair cells are being demonstrated at both ends of the section, due to the curvature of the cochlea, the center part of the micrograph is showing the cut through the area of myelinated fibers medial to the habenula perforata

The large sections are mounted on one hole grids using procedures described by Rowley & Moran (1975) Ribbons are picked up from the boat on open one hole grids that have been dipped in Formvar to cover the grid but not the slot The ribbon of sections centers itself in the drop of water covering the slot of the grid Grids with sections are then placed on the formvar covered holes of a small aluminium or stainless steel rack (see above reference) and allowed to dry The procedure provides large flat sections that are virtually wrinkle free (Fig 9) Grids are stained as desired using conventional methods with times appropriate for the embedding media Providing good Form var is used, and care is taken in casting the films, specimens may be examined at highest magnification and grids can be taken in and out of the TEM without damage to the films

DISCUSSION

The tremendous amount of ultrastructural information available from a single grid of wrinkle free specimens of the size provided with this technique is immediately obvious to the electron microscopist Information on a

single section can cover the disposition of the ultrastructure of sensory cells basilar membrane, tectorial membrane blood and nerve supply, and supporting cells Bohne (1972) and Spoendlin & Brun (1974) have demonstrated techniques for embedding the entire cochlea before bone removal to prevent distortion of cochlear structure during dissection Procedures for obtaining large specimens described in this paper are compatible with their procedures

Any technique that permits the assessment of relative condition of different structures is valuable not only for defining pathology but also for separating pathology from normal variability We can recommend these procedures to inner ear investigators with such interests

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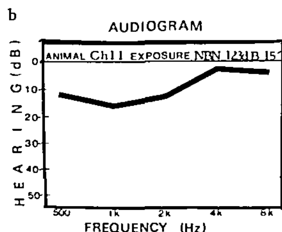
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a

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COCHLEA

HOW

LEFT

I	0	0	1	1	17	1	0	0	1
O_1	34	3	3	3	43	3	6	3	4
O_2	5	2	4	0	28	5	1	1	2
O_3	5	17	15	3	17	2	0	3	1

RIGHT

I	0	0	1	0	0	0	1	0	2
O_1	3	5	0	0	0	7	1	4	1
O_2	0	2	1	0	1	7	0	7	0
O_3	3	1	3	0	1	3	0	2	0

APEX BASE

MISSING HAIR CELLS

Fig 1 (*a*) Low power light micrograph showing lesion in cochlea of a chinchilla exposed to a 1 kHz pure tone at 120 dB for 15 min (*b*) Corresponding behavioral audiogram and cochleogram showing missing hair cells in the exposed (*left*) and unexposed cochleas (from Hunter Duvar & Bredberg 1974) *BC*=Boettcher cells *HC*=Hensen cells *CC* Claudius cells *OC*=organ of Corti *MNF*–myelinated nerve fibers picture width (pw)=650 μm

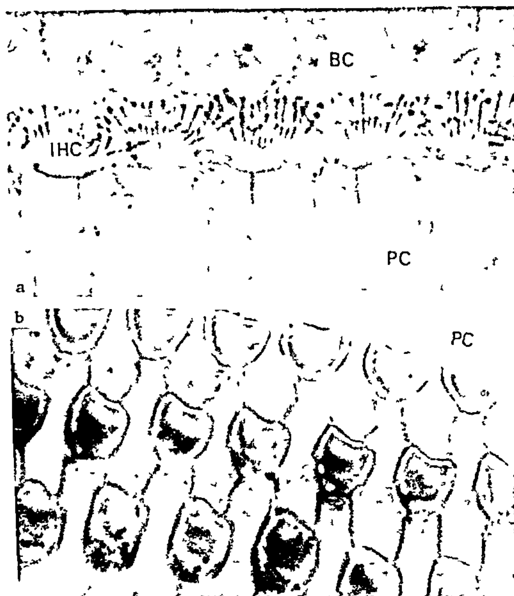


Fig 2 Light m crographs of cochlear specimens demonstrating resolution obtained when specimens are embedded in plastic slides and viewed with a phase microscope (*a*) shows inner hair cell cilia (*IHC*) and headplates of inner pillar cells (*PC*) (*b*) focus at level of cuticular plates of outer hair cells *BC*=Border cells pw *a*=40 μm *b* 40 μm

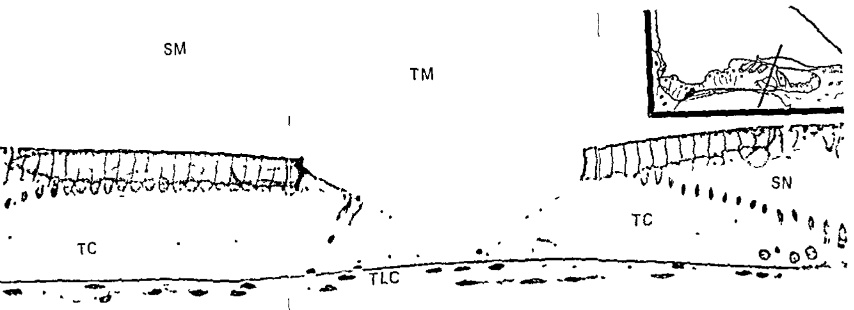


Fig 3 Light micrograph of a 1 μm *thick* section perpendicular to the basilar membrane through the *lesion area* shown in Fig 1 Orientation is as shown in the inset The tunnel of Corti has been sealed by supporting cells at both ends of the lesion *SM*=scala media, *TM*=tectorial membrane, *PC*=pillar cells *TC*=tunnel of Corti *TLC*=tympanic layer cells, *SN*=space of Nuel pw=300 μm

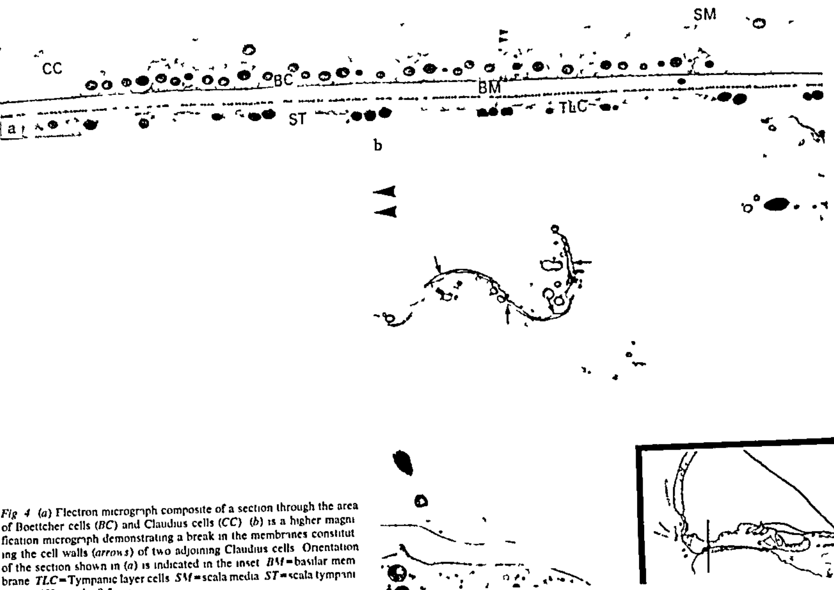


Fig. 4. (*a*) Electron micrograph composite of a section through the area of Boettcher cells (*BC*) and Claudius cells (*CC*). (*b*) is a higher magnification micrograph demonstrating a break in the membranes constituting the cell walls (*arrows*) of two adjoining Claudius cells. Orientation of the section shown in (*a*) is indicated in the inset. *BM* = basilar membrane. *TLC* = Tympanic layer cells. *SM* = scala media. *ST* = scala tympani. pw. *a* = 450 μm. *b* = 8.5 μm.

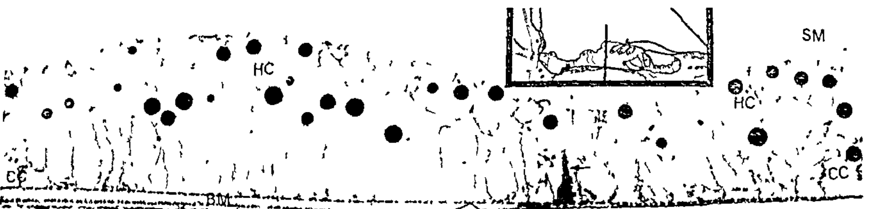


Fig 5 Transmission electron micrograph composite taken in the *scanning mode*, of a section through the Hensen cells (*HC*) of the lesion area *shown in* Fig 1 Overgrowth of cells in the damaged area as well as *degenerating cells* (*arrow*) is shown Insert shows section orientation *SM*=scala media *BM*=basilar membrane *CC*=Claudius cells pw=350 µm

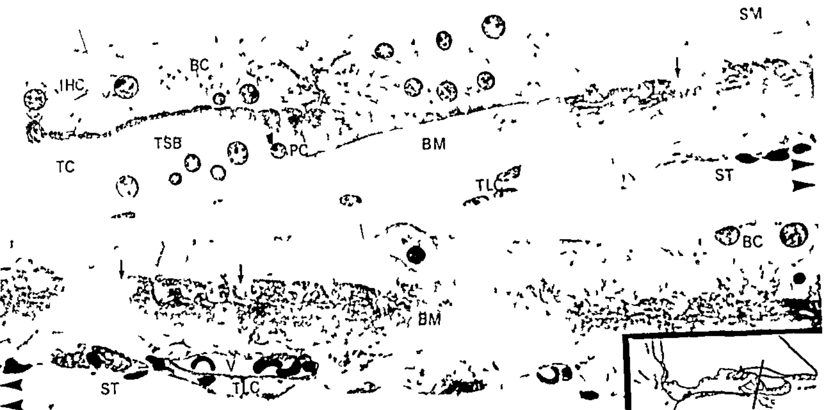


Fig 6 Electron micrograph composite through inner hair cell area of specimen shown in Fig 1 The lower picture is the right half of the micrograph Inner hair cells (*IHC*) have been sectioned tangentially rather than lengthwise due to the cutting angle which is shown in inset The empty or nearly empty habenula perforata (*arrows*) are consistent with the lack of nerve fibers seen in Fig 1 Giant axons (*A*) are seen at the bases of inner hair cells at the borders of the main lesion area Blood vessels (*V*) appear normal *SM*=scala media *TC*=tunnel of Corti *TSB*=tunnel spiral bundle *BM*=basilar membrane *PC*=pillar cells *TLC*=tympanic layer cells *ST*=scala tympani *BC*=border cells pw=300 μm overall

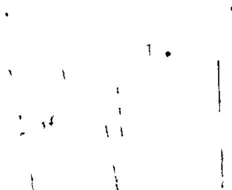


Fig 9 A low power light micrograph shows a ribbon of ¾ mm long sections mounted on a Formvar coated grid prior to viewing with the TEM Sections are generally free of wrinkles and definition of organ of Corti structures can be detected pw–3 mm



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Proteinsynthese der Cochlea und Beschallung

Autoradiographische Untersuchungen

VON

WILFRIED RICHRATH

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Proteinsynthese der Cochlea und Beschallung

Autoradiographische Untersuchungen

VON

WILFRIED RICHRATH

Aus der HNO-Klinik der Westfälischen Wilhelms-Universität Münster/Westfalen (D 4400)
(Direktor: Prof. Dr. H. Feldmann)

MÜNSTER 1977

Herrn Prof. Dr. Karl Mundnich
zum 70. Geburtstag gewidmet

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1. Einleitung

Nach den ersten Berichten über die Histopathologie des Lärmschadens vermutete Wittmaack (1907) eine Störung im Zellstoffwechsel, deren Ursache trotz zahlreicher Untersuchungen nicht aufgedeckt worden ist.

Die Erhaltung von Struktur und Funktion einer Zelle ist von Proteinen abhängig. Histochemische Beobachtungen nach Häutchenpräparation des Cortischen Organs (Beck und Michler, 1960, Vinnikov und Titova, 1963) lassen annehmen, daß die Proteinsynthese in den Zellen der Cochlea zumindest durch starke Schallreize schnell reduziert wird. Cytophotometrisch und mikroradiographisch zeigten Nervenzellen des Ganglion spirale nach intensiver Reizung eine Abnahme der Proteinkonzentration (Hamberger und Hydén, 1945, Hammer, 1956, 1958), nach Belastung mit physiologischen Reizen ließen sie jedoch auch relativ früh Zeichen der Regeneration erkennen (Hamberger und Hydén, 1945). Autoradiographisch wurde nach Schallexposition sowohl eine Zunahme (Koburg und Hempel, 1965) wie eine Abnahme (Anichin, 1970) der Aminosäureinkorporation in Ganglien- und Haarzellen beschrieben. Bei der Beurteilung dieser widersprüchlichen Ergebnisse ist die unterschiedliche Versorgungslage der einzelnen Gewebe des Innenohres zu berücksichtigen. Zentrale, modiolusnahe und periphere labyrinthkapselnahe Bereiche besitzen ein Gefäßsystem, während das Cortische Organ beim metabolischen An- und Abtransport weitgehend auf die Peri- und Endolymphe als Medium angewiesen ist. Dementsprechend ist der Stofftransport zu den Sinneszellen deutlich verzögert gegenüber dem zu Ganglienzellen oder zur Stria vascularis (Meyer zum Gottesberge und Plester, 1961). Neben dieser anatomischen Eigenheit hängt die metabolische Versorgung des Cortischen Organs während der Beschallung zusätzlich von der Reizintensität ab, da sich die Halbwertszeit der Perilymphe oberhalb der kritischen Intensität von 90 db Schalldruck verdoppelt (Schnieder 1974).

Langzeituntersuchungen zur Proteinsynthese der Cochlea in Ruhe oder nach Beschallung fehlen ebenso wie Angaben über Windungsunterschiede bei den Sinneszellen. In der vorliegenden Mitteilung wird über autoradiographische Ergebnisse zur Kinetik des Aminosäureeinbaues unter verschiedenen Schallbedingungen berichtet. Da für Stoffwechseluntersuchungen *in vitro* die Isolierung der einzelnen Zelltypen des Innenohres in ausreichender Menge kaum möglich ist, sind biochemische Untersuchungen an der Cochlea weitgehend auf morphologische Methoden angewiesen. Unter diesen gewährleisten elektronenmikroskopische Präparationsverfahren eine leichter reproduzierbare morphologische Erhaltung und eine geringer schwankende Volumenänderung als die bisher übliche Schneckenentkalkung mit anschließender Paraffineinbettung. Da autoradiographisch die Aktivität pro Volumeneinheit erfaßt wird und sich unterschiedliche Volumenänderungen durch Kunststoffeinbettung verringern lassen, nimmt dieser methodische Fehler beim Vergleich der Silberkorndichten einer größeren Anzahl von Präparaten ebenfalls ab. Für die quantitative Auswertung zeigte sich, daß reflexionsphotometrische Silberkornbestimmungen, die bisher nur auf Einzelzellautoradiogramme anwendbar waren, nach Einengung der Beleuchtungsapertur auch an Autoradiogrammen von kunststoffeingebetteten Geweben durchgeführt werden können.

2. Material und Methoden

2.1 VERSUCHSTIERE

Verwendet wurden pigmentierte Meerschweinchen beiderlei Geschlechts aus einem händlereigenen Inzuchtstamm. Nach dem Körpergewicht von 100 bis 200 g handelt es sich um juvenile Tiere (Reid und Briggs, 1953). 12 Stunden vor Versuchsbeginn wurde allen Tieren die Nahrung entzogen (Wasser ad lib.).

2.2 VERSUCHSANORDNUNGEN

2.2.1. Kurzversuch, Seitenvergleich unter Beschallung

In Narkose mit Chlorpromazin (14 mg/kg, i.p.) und Pentobarbitalnatrium (33 mg/kg, i.p.) wurde 12 Meerschweinchen die rechte Ohrmuschel abgetragen und das Ohr (Beschallung) mit 85 db weißem Rauschen bis zur Dekapitation 1, 2, 3 und 4 Stunden nach der intraperitonealen Injektion von 4,5-^{3}H-DL-Leucin (5 mCi/150 g Körpergewicht, spez. Aktivität 19,4 Ci/mMol, Radiochemical Centre, Amersham, England) beschallt. Hierzu war an der rechten Bulla ein Kunststoffstutzen mit Histacryl fixiert und über einen Schlauch mit der Schallquelle verbunden. Das linke Ohr diente als Kontrolle.

2.2.2. Langzeitversuch zur Kinetik

24 Tiere erhielten 1/2 Stunde vor Versuchsbeginn ein Standardfutter bei Nahrungsentzug während der restlichen Versuchszeit (Wasser ad lib.). Sie wurden in der Camera silens bis zur Dekapitation 1, 2, 3, 4, 8 und 24 Stunden nach intraperitonealer Injektion von ^{3}H-DL-Leucin (sonstige Angaben wie im Kurzversuch) mit einem weißen Rauschen von 35 db beschallt. Vor der Injektion war eine Hälfte der Tiergruppe über 12 Stunden weißem Rauschen von 35 db (Kontrolle), die andere von 85 db (Beschallung) ausgesetzt. Der Lautsprecher war aus 2 m Entfernung auf die Mitte eines weitmaschigen Drahtkäfigs gerichtet. Die Bandbreite des Rauschgenerators (Typ 1402, Brüel und Kjoer) betrug 20 Hz bis 20 kHz. Tonfrequenzspektrographisch (Typ FNA, Rhode und Schwarz) ergab sich eine Linearität bis 20 kHz.

2.3 PRÄPARATIONSMETHODEN

Beiderseits wurde innerhalb von 5 Minuten nach Dekapitation in Äthernarkose vom runden Fenster bis zur Schneckenspitze ein schmaler Knochenstreifen ausgebrochen und das Innenohr mit 2,5 %iger, gepufferter Glutaraldehydlösung (pH 7,3) durchspült. Nach Isolierung vom Schädel wurden die zurechtgeschnittenen Felsenbeine in der Glutaraldehydlösung insgesamt 2 Stunden lang fixiert und bei Zimmertemperatur über 24 Stunden in mehrfach gewechseltem Sorensen-Puffer ausgewaschen. Pro Intervall des Langzeitversuches wurde je ein Felsenbein aus Kontrolle und Beschallung eine Stunde lang in 1-%iger Osmiumsäure nachfixiert. Unter Einhaltung konstanter Bedingungen erfolgte in üblicher Weise die Einbettung in Epon. Nach dem Auspolymerisieren wurde die Knochenkapsel der Schnecke freigelegt und nach 2-stündiger Einwirkung von 5-%iger Trichloressigsäure abgezogen.

Für elektronenmikroskopische Untersuchungen wurden zentrale Scheiben der osmiumfixierten Schnecken in die vier Win-

dungen zerlegt und hellgelbe Dünnschnitte nach Kohlebedampfung mit einer Monolage der Photoemulsion Kodak L4 beschichtet. Die Expositionszeit betrug ca. 90 Tage, dem Entwickeln und Fixieren schloß sich eine Nachkontrastierung mit Bleizitrat an.

Für die Lichtmikroskopie wurde von den übrigen Schnecken ohne weitere Entkalkung mit Glasmessern ca. 3 μm dicke Schnitte parallel zum Modiolus und längs des früheren Bruchstreifens hergestellt und für Autoradiogramme auf Objektträgern zusätzlich mit einer Celloidinhaut von ca. 100 Å Dicke belegt. Durch Tauchen wurde mit der 1:1 verdünnten Fotoemulsion Agfa-Gaevert Nuc 715 überschichtet. Nach Exposition über 4 bzw. 5 Monate bei 4° Celsius unter Lichtabschluß und Zugabe von Silicagel als Trockenmittel wurden alle Präparate der jeweiligen Versuchsreihe in einem Halter gemeinsam entwickelt, fixiert, gewässert (Rezepte der Fa. Agfa Gaevert) und nach dem Trocknen ungefärbt in Harz vom Brechungsindex $n_{D^{20}} = 1.53$ eingedeckt (Deckglasdicke 0,18 mm ± 10%).

2.4 UNTERSUCHUNGS- UND AUSWERTUNGSMETHODEN

Die elektronenmikroskopischen Untersuchungen wurden mit dem Elmiskop I (Fa. Siemens) durchgeführt und Vergrößerungen von 3000–8000× benutzt[1].

Lichtmikroskopisch wurden fast alle Gewebe der Basalwindung im Langzeitversuch zusätzlich innere und äußere Haarzellen aller Windungen ausgewertet. Über schwach markierten Zellen, wie im Cortischen Organ, konnte die Silberkornzahl nur visuell mit einem Okularnetzmikrometer bestimmt werden (Phasenkontrast. Ölimmersion 100×, Okular 20×, objektbezogene Einzelfeldgröße 16 μm^2), über stark markierten Zellen z. T. reflexionsphotometrisch (s. Anhang). Ausgezählt bzw. photometriert wurden vier Autoradiogramme bis zu einer Gesamtzahl von 100 Einzelwerten pro Zelltyp und Felsenbein, für Kerne der Haarzellen dagegen alle Anschnitte von wenigstens 10 bis 15 Autoradiogrammen jeder Schnecke. Das Meßergebnis von guten Kernanschnitten wurde mit Hilfe des jeweiligen Durchmessers auf Silberkörner pro 16 μm^2 umgerechnet. Zusätzlich wurde der Kerndurchmesser aller Haarzellen an ca. 30 Nachschnitten jeder einzelnen Schnecke des Langzeitversuches lichtmikroskopisch mit einem Strichmikrometer bestimmt.

2.5 STATISTISCHE SICHERUNG

Die Mittelwerte der Silberkorndichte über den Kernen der Ganglienzellen und dem Cytoplasma aller Zellen wurde mit Hilfe eines linearen Modells[2] einer Varianzanalyse unterzogen. Es wurde angenommen, daß die Effekte von Felsenbeinen, Behandlungen (Kontrolle und Beschallung) sowie Markierungszeiten unabhängig und die Mittelwerte der vier je Cochlea und Zelltyp ausgewerteten Autoradiogramme normal verteilt sind. Die Streuungszerlegung erfolgte mittels Rechenprogrammen der Firma IBM (Application Program H20-0205-3, 1968), die Tabelle der vollständigen Varianzanalyse wurde durch Zusammenfassen von Variabilitätsursachen auf die Streuungskomponenten des linearen Modells reduziert. Die mittleren Quadrate von Markierungsdauer, Beschallung und deren Wechselwirkung wurden mit der *F*-Verteilung gegen den Versuchsfehler und Unterschiede zwischen Mittelwerten der einzelnen Markierungszeiten mit dem Tukey-Test überprüft (vgl. Weber, 1967). Vom letzteren sind die *p*-Werte als Symbole in den Tabellen angegeben:

$\varnothing = p > 0{,}05$, $+ = p \leq 0{,}05 > 0{,}01$, $\ddagger = p \leq 0{,}01$

[1] Herrn Prof. H. G. Fromme danke ich für die Übernahme der autoradiographischen Präparation der Dünnschnitte und Herrn Prof. Dr. G. Pfefferkorn, Direktor des Institutes für Medizinische Physik der Universität Münster/Westf., für die Erlaubnis, das Mikroskop zu benutzen.

[2] Für die Erstellung und Interpretation des statistischen Modells danke ich Herrn Dipl.-Math. E. Hultsch, Institut für Medizinische Informatik und Biomathematik der Universität Münster/Westfalen.

dıc Gewebe ınnerhalb eıner Schnecken-n ıung (Tab II) wurde der Mıttelwertsver-ıch fur Poısson Verteılungen, fur Zellkern-urchmesser unter Annahme eıner Normal-verteılung der t-Test durchgefuhrt (vgl Sachs, 1972) Dıe Symbole fur p-Werte entsprechen den Angaben zum Tukey-Test

3. Ergebnisse

3.1. MORPHOLOGIE UND AUTORADIOGRAMME

3.1.1. Lichtmikroskopie

Die außerst empfindlichen Zellen des Cortischen Organs sind in den vier Windungen gut erhalten (Abb. 1). Stütz- und Sinneszellen sind muhelos zu differenzieren. Vereinzelt erkennt man Vacuolen in den Haarzellen. Als Artefakt ist die Tectorialmembran fast immer von der Lamina reticularis abgehoben, die Reißnersche Membran haufig gerissen. Das Epithel des Sulcus internus und externus zeigt hohe kubische Zellen mit nur sparlichem Ergastoplasma. Besonders plastisch treten die Bindegewebsfasern des Ligamentum spirale hervor. Morphologische Unterschiede zwischen Beschallung und Kontrolle finden sich nicht.

Die Autoradiogramme der Innenohrschnekken lassen uber den einzelnen Zellen eine unterschiedliche Silberkorndichte erkennen, die mit den Meßergebnissen ubereinstimmt. Gelegentlich sieht man uber den Nervenendigungen und dem basalen Bereich der außeren Haarzellen eine Anhaufung von Silberkornern. Die hochste Silberkorndichte teils als Konglomerate zeigen angeschnittene Nucleoli der Ganglienzellen, deren Karyo- und Cytoplasma scheint gleichmaßig markiert. Bei allen anderen Zellen sind die Kerne deutlich starker markiert als das Cytoplasma. Eine inhomogene Verteilung der Silberkorner zeigt sich uber der chromophilen Schicht der Stria vascularis und den Sulcusepithelien. Bei letzteren hangt die Anzahl der Silberkorner von der Dichte des Ergastoplasmas ab, in optisch leeren Raumen tritt fast keine Markierung auf. Die Silberkorndichte der Sulcusepithelien betragt durchschnittlich nur 10% von der über Ganglienzellen und liegt damit an der unteren Grenze aller Innenohrzellen. Uber angeschnittenen Gefaßlumina finden sich mehr Silberkorner als uber dem Hintergrund. Dabei besteht uber Leukozyten eine Anreicherung, die bei langerer Versuchsdauer zunimmt. Erythrozyten und optisch leere Räume erscheinen diffus markiert. Die Tectorialmembran unterscheidet sich in ihrer Silberkorndichte kaum von der des Hintergrundes. Der Stamm des Nervus acusticus ist in allen Versuchen markiert, im Vergleich zu den Axonen überwiegt deutlich die Silberkorndichte der Schwannschen Zellen. In der lichtmikroskopischen Ubersicht lassen sich für den einzelnen Zelltyp weder zwischen Kontrolle und Beschallung noch zwischen verschieden langen Versuchszeiten auffallende Unterschiede in der Markierungsdichte erkennen.

3.1.2. Elektronenmikroskopie

Pro Intervall des Langzeitversuches wurde je ein Felsenbein aus der Kontroll- und Beschallungsreihe für elektronenmikroskopische Autoradiogramme aufgearbeitet. Die morphologische Erhaltung submikroskopischer Strukturen ist ausreichend, der Bildkontrast jedoch relativ schwach. Die verschiedenen Gewebe der Cochlea eines Windungsquerschnittes unterscheiden sich bei insgesamt niedriger Hintergrundsmarkierung betrachtlich durch ihre Silberkorndichte. Trotz langer Expositionszeit zeigen nur die Zellen des Ganglion spirale cochleae und der Stria vascularis eine ausreichend starke Markierung. Wegen der von Schnitt zu Schnitt des gleichen Präparates zu stark schwankenden und wegen der unzureichenden Markierung der Sin

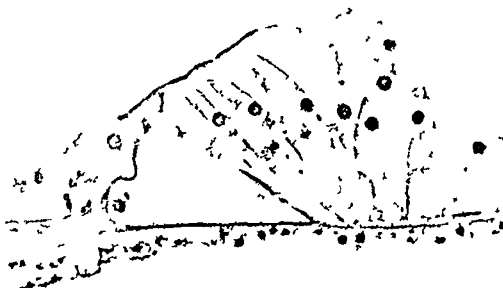


Abb 1 Autoradiogramm des Corti schen Organs aus der 2 Schneckenwindung vom Meerschweinchen 2 Stunden nach Beschallung und ^{3}H Leucin Eponeinbettung Phasenkontrast Objekt v 40× Gesamtvergrößerung ×480

mußte auf eine quantitative Auswertung verzichtet werden

Im Cytoplasma von Ganglienzellen (Abb *a*) liegen die Silberkorner weitgehend gleichmäßig über dem rauhen endoplasmatischen Reticulum sowie über Vakuolen des Golgi Komplexes Anfanglich zeigen Mitochondrien Ursprungskegel und Axone nur gelegentlich eine autoradiographische Reaktion diese nimmt hier mit langerer Versuchszeit ebenso zu wie uber den Neurotubuli und Neurofilamenten (Abb 2*b*) Im Karyoplasma zeigen angeschnittene Nucleoli die größte Silberkorndichte wahrend die restliche Markierung nahe dem Ubergang von Eu zu Heterochromatin sowie über der Kernmembran liegt Dieses Verteilungsmuster bleibt zu allen Versuchszeiten identisch

Relativ hoch ist die Markierungsdichte der Schwannschen Zellen Auch über deren Zellkernen liegen die Silberkörner bevorzugt im grenznahen Bereich beider Chromatinformen Die den Hullzellen zugehorigen Myelinscheiden um Ganglienzellen und Axone zeigen von der 1 Stunde an eine ^{3}H Leucininkorporation Es ist jedoch wegen des begrenzten Auflosungsvermogens der Autoradiographie nicht möglich die Radioaktivitat bestimmten Strukturen des Myelinmantels zuzuordnen

Uber den außeren Haarzellen (Abb 3*a*) scheint die Inkorporation in das zellwandnahe aus flachen Zisternen aufgebaute endoplasmatische Reticulum sowie in angrenzende und infranukleare Mitochondrien zu uberwiegen wahrend der subapikal gelegene Hensen Korper ganz selten markiert ist (Abb 3*c*) Bei inneren Haarzellen (Abb 3*b*) liegen die Silberkorner eher uber das ganze Cytoplasma verteilt Die Sinneszellhaare zeigen keine autoradiographische Reaktion dagegen haufig die Kuticularplatte und die Reticularmembran Im Synapsenbereich lassen sich bei nur geringer Inkorporation keine auffallenden Unterschiede zwischen afferenten und efferenten Nervenendigungen erkennen

Bei Pfeilerzellen ist der Bereich mit Fibrillen bevorzugt markiert Dies zeigt sich noch deutlicher über der Basilarmembran Deren Grundsubstanz in der Pars pectinata ist weitgehend frei wahrend die Filamente wie die Pars tecta einen deutlichen Aminosaureeinbau erkennen lassen der oberhalb des Spiralgefaßes leicht

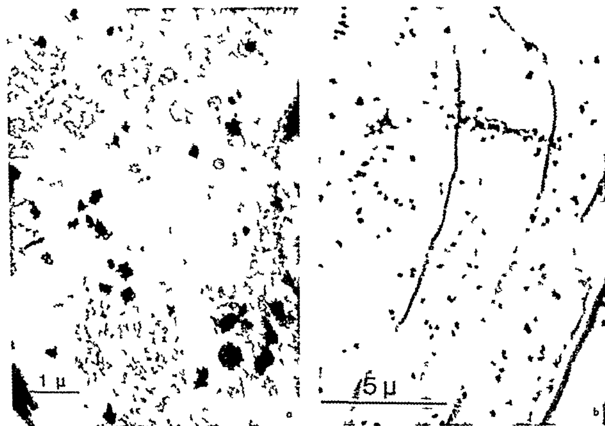


Abb 2 Elektronenmikroskopische Autoradiogramme vom Ganglion spirale cochleae des Meerschweinchens 4 Stunden nach ³H DL Leucin Emulsion L 4

zunimmt Die fibrillaren Strukturen der Tectonalmembran sind dagegen nicht starker als der Hintergrund markiert

Bei den zwei Zellschichten der Reißnerschen Membran sind die Silberkorner fast ganz den Epithelien der Endolymphseite zuzuordnen da die Endothelien der perilymphatischen Oberflache nur gelegentlich eine Markierung der stark ausgezogenen dunnen Cytoplasmaschicht zeigen

Von den Zellen der Stria vascularis zeigt das chromophile Epithel die starkste Inkorporation was auch fur deren zahlreiche Zellfortsatze bestehen bleibt die sich in tieferen Schichten mit Auslaufern der geringer markierten chromophoben Zellen verflechten Vereinzelte Kapillarlumina sind durch dicht aneinander gelagerte Erythrozyten ausgefullt die Haufigkeit dieses Phanomens scheint jedoch bei Kontrolle und Beschallung gleich

3 2 ERGEBNISSE DER MESSUNGEN

3 2 1 Silberkorndichte im Kurzversuch Seitenvergleich unter Beschallung

Jeder Mittelwert stammt von drei Felsenbeinen verschiedener Tiere deren rechtes Ohr bis zu 4 Stunden in Narkose beschallt wurde das linke Ohr dient als Kontrolle In jedem Schnitt erlaubt eine große Anzahl von Ganglienzellen die Abgrenzung von Karyo und Cytoplasma und deren getrennte Auswertung wahrend bei Sulcusepithelien Haar und Deiterschen Zellen wegen der geringen Zahl



Abb 3 Elektronenmikroskopische Autoradiogramme von einer außeren (*a*) und inneren (*b*) Haarzelle der Meerschweinchencochlea, Photomontage wegen Zellgroße ubrige Angaben wie Abb 2

Kernanschnitten nur das Cytoplasma ausgezahlt ist Dagegen ist bei der Stria vascularis und den Fibrozyten des Ligamentum spirale wahrend der Auswertung keine strenge Trennung zwischen Zellkernen und Cytoplasma durchgefuhrt worden

Uber dem Cytoplasma der linksseitigen Innenohrzellen (Kontrolle) steigt die Silberkorndichte bis zur 2 Stunde an, bleibt für eine Stunde auf ungefahr gleicher Hohe und fallt zur 4 Stunde wieder stark ab (Abb 4) Der Kurvenverlauf ist auch fur die Gewebe, bei denen keine strenge Trennung zwischen Cytoplasma und Kern durchgefuhrt wurde, iden-



Abb. 3c. Elektronenmikroskopisches Autoradiogramm einer äußeren Haarzelle mit Hensenkörper. Übrige Angaben s. Abb. 3

tisch. Bezogen auf die Silberkorndichte über dem Cytoplasma von Ganglienzellen (= 100%) ergibt sich für das einzelne Gewebe von der 1. bis 4. Stunde eine weitgehend gleiche prozentuale Relation. Die Meßwerte über allen Zellen des rechten, beschallten Ohres liegen zu jeder Versuchszeit über den Kontrollwerten. Nach der Varianzanalyse verlaufen die Kurven von Kontrolle und Beschallung parallel. Die Mittelwertsunterschiede lassen sich bei schwächer markierten Geweben statistisch sichern und zeigen für Haar-, Deitersche Zellen, Sulcusepithelien sowie Fibrozyten eine stärkere Inkorporation von ^{3}H-Leucin auf der beschallten Seite.

Bei Kontrollen bleibt die Silberkorndichte der Ganglienzellkerne zu allen Versuchszeiten deutlich unter der des Cytoplasmas. Unter der Beschallung nimmt die Inkorporation zu. Die Zunahme bei Ganglienzellen und bei der Stria vascularis ist jedoch statistisch nicht zu sichern.

3.2.2 Silberkorndichte im Langzeitversuch. Kinetik

3.2.2.1 *Gewebe der Basalwindung*

Im Langzeitversuch mit wachen, hungernden Meerschweinchen wird das Markierungsverhalten von Innenohrzellen bei 35 db (Kontrolle) mit dem von Zellen nach Vorbeschallung mit 85 db (Beschallung) weißem Rauschen bis 24 Stunden nach ^{3}H-DL-Leucin-Injektion verglichen. Für jeden Mittelwert sind drei Felsenbeine von 2 Tieren ausgewertet und die durchschnittliche Silberkorndichte für Kontrolle und Beschallung eingetragen (Abb. 5a und 5b, 6). Auch hier ist eine getrennte Auswertung für Kern und Cytoplasma nicht bei allen Zelltypen möglich. Bei Ganglienzellen sowie Haarzellen sind Kern und Cytoplasma getrennt gemessen. Die Werte für Hensen- und Deitersche Zellen, die Sulcus- und Limbusepithelien beziehen sich nur auf das Cytoplasma. Bei den restlichen Geweben ist eine genaue Trennung zwischen Kern und Cytoplasma nicht durchgeführt, es überwiegt jedoch bei der Auswertung der Autoradiogramme der cytoplasmatische Flächenanteil.

Im Cytoplasma steigt bei Kontrollen die Silberkorndichte über fast allen Zelltypen der Basalwindung bis zur 3. Stunde an (Abb. 5a). Lediglich über der Stria vascularis, den Zellen am Boden der Scala vestibuli, dem äußeren Sulcusepithel und der Reißnerschen Membran ist schon nach 2 Stunden ein Gipfel erreicht. 4 und 8 Stunden nach der Injektion des ^{3}H-Leucins nimmt die Markierungsdichte über den meisten Geweben wieder ab, ohne jedoch die 1-Stunden-Werte zu unterschreiten. Abweichend davon fallen die Meßwerte über dem Cytoplasma der Ganglienzellen nach 8 Stunden auf ein deutlich unter dem Anfangswert liegendes Minimum. Nach 8 Stunden steigt die Silberkorndichte über jedem Gewebe wieder an und erreicht über fast allen erst nach 24 Stunden die absolut höchsten Werte. Damit zeigen die Gewebe einen biphasischen Verlauf im zeitlichen Verhalten der Markierungsdichte, was besonders deutlich beim Cytoplasma der Ganglienzellen zu erkennen ist.

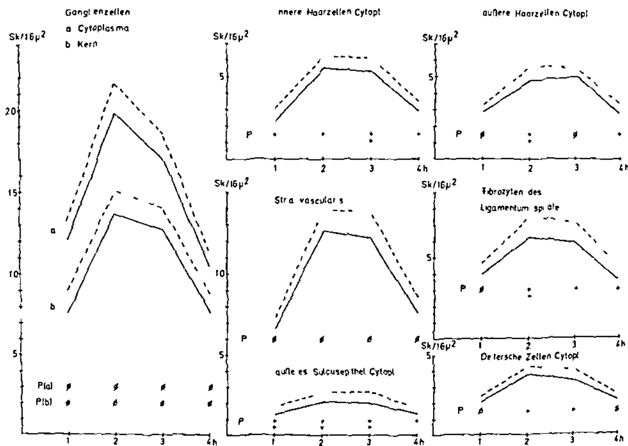


Abb 4 Kurzversuch Graphische Darstellung der Silberkorndichte uber Zellen der Basalwindung Zur Aufteilung in Cytoplasma und Zellkern siehe Ergebnisse Abszisse Zeitdauer nach intraperitonealer Injektion von ³H DL Leucin und rechtsseitiger Beschallung mit 85 db Rauschen in Narkose Ordinate mittlere Silberkorndichte pro 16 μm² —=Kontrolle (linke Seite) - =Beschallung (rechte Seite) Symbole fur Mittelwertsunterschiede vergl Methoden

Die Kurven der Silberkorndichte nach Beschallung beginnen fur das Cytoplasma aller Zellen mit hoheren Werten als bei Kontrollen (Abb 5*a*) Fur Ganglienzellen, die Reißnersche Membran und das Limbusepithel ist das erste Maximum bereits bei einer Stunde erreicht, wahrend andere Zelltypen nach 2 Stunden die hochsten Meßwerte aufweisen Damit liegt das erste Maximum der Silberkorndichte nach Beschallung vor dem der Kontrollen Die Vorverlagerung von Extremwerten wird mit der geringsten Silberkorndichte nach 4 Stunden gegenüber den Kontrollwerten noch deutlicher, zumal bei beschallten Ohren die Ausgangswerte fur alle Zelltypen unterschritten werden Nach 8 Stunden sind durch einen starken Anstieg die Kontrollwerte erreicht oder uberschritten, nach 24 Stunden bestehen mit Ausnahme bei Ganglienzellen nur noch geringe Unterschiede gegenüber Kontrollen

Die Markierungsdichte uber Kernen der Ganglienzellen zeigt bei Kontrollen (Abb 5*b*) einen dem Cytoplasma weitgehend identischen Verlauf, wobei die Silberkorndichte insgesamt nur ca 70% von der des Cytoplasmas

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Abb 5a Langzeitversuch Zeitlicher Verlauf der Silberkorndichte uber Cytoplasma und Zellen (s Text) der Gewebe in der Basalwindung Abszisse Stunden nach intraperitonealer Injektion von ³H DL Leucin Ordinate mittlere Silberkorndichte pro 16 μm² —=Kontrolle (12 Std vor u bis 24 Std nach der Injektion 35 db) =Beschallung (12 Std vor der Injektion 85 db danach bis 24 Std 35 db) Symbole fur Mittelwertsunterschiede s unter Methoden

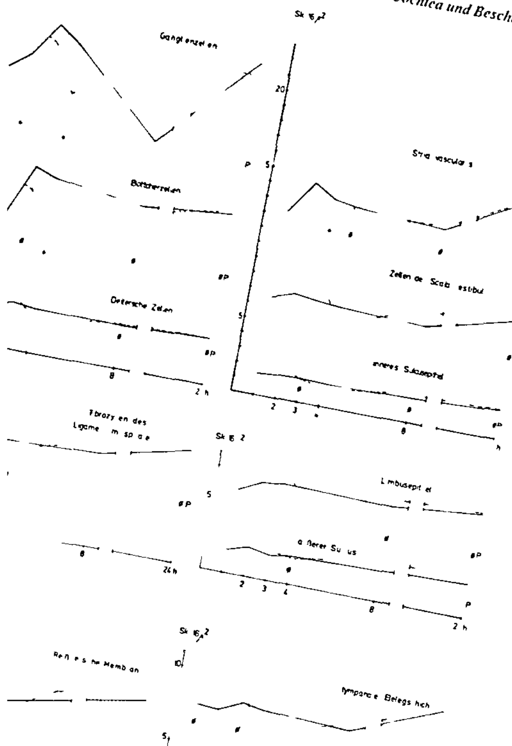

Böttcherzellen
Deitersche Zellen
20
5
2 h
24 h
2 h

Tabelle II *Gegenüberstellung von Relationen der Silberkorndichte und des RNA-Gehaltes der verschiedenen Innenohrgewebe der Basalwindung*

Spalten 1–2 Relation der Silberkorndichte im Cytoplasma von Kontrolle (K) und Beschallung (B) nach Eponeinbettung Zum Vergleich mit Spalte (5) 2 Std nach Leucininjektion Cytoplasmamarkierung der Ganglienzellen = 100%
Spalten 3–4 Prozentuale Zu oder Abnahme der Silberkorndichte über dem Cytoplasma nach Beschallung im Vergleich [illegible] Gewebe (4)

S[illegible] esberge 1961
Koburg und Plester 1962)
Spalte 6 Relationen der cytophotometrisch gemessenen RNA Konzentrationen (Kraus 1970)
Spalte 7 Quotient aus Eiweiß Umsatz ohne Entkalkung (Spalte 1) und RNA Konzentration nach Entkalkung (Spalte 6)

	Eiweiß Umsatz 2h nach ³H Leucin ohne Entkalkung (%)		Zu oder Abnahme von B zu K (%)		Eiweiß-Umsatz 2h nach ³H Leucin nach Entkalkung (%)	RNA Gehalt (Gallocyanin chromalaun) nach Entkalkung (%)	Eiweiß-Umsatz pro RNA Gehalt
	K (1)	B (2)	2h (3)	1 Max (4)	(5)	(6)	(7)
Ganglienzellen (Ggl spirale)	100	100	*+ 9*	*+ 1*	100	100	1,0
Stria vascularis (chromophobe Schicht)	74	75	*+10*	*+10*	71	59	1,3
Cortisches Organ							
Äußere Haarzellen	17	19	*+24*	*+15*	53	17	1,0
Innere Haarzellen	26	27	*+13*	*+15*	53	21	1,2
Deitersche Zellen	14	18	*+39*	*+19*	50	12	1,2
Hensen Zellen	20	23	*+23*	*+20*	50	9	2,2
Böttcherzellen	45	53	*+28*	*− 7*	81	11	4,0
Äußeres Sulcusepithel	10	13	*+40*	*+40*	42	15	0,7
Inneres Sulcusepithel	8	10	*+31*	*+31*	42	15	0,5
Fibrozyten des Lig spirale	31	40	*+38*	*+32*	68	65	0,5
Reißnersche Membran	59	59	*+ 7*	*+13*	53	40	1,5
Tympanale Belegschicht	39	49	*+36*	*+23*	54	65	0,6
Limbusepithel	33	36	*+20*	*+30*	35	38	0,9
Zellen der Scala vestibuli	36	42	*+26*	*+26*	58	34	1,1

zur jeweiligen Ganglienzellmarkierung einander Die Werte der Spalte 1 stimmen weitgehend mit der cytophotometrisch mit Hilfe der Gallocyanin Chromalaun-Färbung gemessenen, relativen RNA Konzentration (Spalte 6) überein, der Quotient aus beiden schwankt um 1 und erreicht lediglich bei Hensen- und Böttcherzellen höhere Werte (Spalte 7)

Nach Beschallung nehmen für die einzelnen Innenohrgewebe die Relationen zur Markierungsdichte der Ganglienzellen nur gering zu (Spalte 2) Dagegen zeigen 2 Stunden nach Applikation von ³H-Leucin Zellen der Cochlea beschallter Tiere eine um durchschnittlich 25% höhere Aminosäureinkorporation als die von Kontrollen (Spalte 3) Beim Vergleich der maximalen Silberkorndichte (Spalte 4) reduziert sich der Durchschnitt auf 19% Für Sulcusepithelien und Fibrozyten des Ligamentum spirale bleibt auch dabei die höhere prozentuale Inkorporation bestehen, für Limbusepithelien ist eine weitere Steigerung festzustellen Bei den übrigen Geweben nehmen die Unterschiede ab

Im Maximum der Silberkorndichte von Beschallung und Kontrolle besteht für Ganglien- und Böttcherzellen kein Unterschied

3.2.3 Zellkerndurchmesser der Haarzellen, Langzeitversuch

Für jede Schneckenwindung des Langzeitversuches ist bis 4 Stunden nach ³H-Leucin-Gabe der Durchmesser gut angeschnittener Kerne

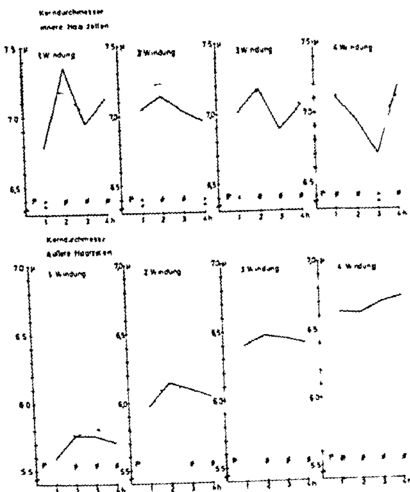


Abb 7 Langzeitversuch (nur 1 4 Stunde) Durchmesser der Ha zellkerne der 4 Schneckenwindu gen Abszisse Stunden nach schallung mit 85 db Rauschen —= Kontrolle ---=Beschallung Ubrige Angaben vergl Abb *5a*

innerer und außerer Haarzellen der Beschal lungs- und Kontrollgruppe gemessen Jeder angegebene Mittelwert basiert auf der Aus wertung von drei Felsenbeinen mit ca 70–150 Einzelwerten

1 Stunde nach Vorbeschallung mit 85 db weißem Rauschen zeigen außere und innere Haarzellkerne einen großeren Durchmesser als die Kontrollen mit 35 db Beschallung den folgenden Versuchszeiten sind kaum n signifikante Unterschiede festzustel (Abb 7)

Die Abnahme des Kerndurchmessers n apikal ist bei inneren Haarzellen nur anged tet, die Zunahme nach apikal bei auße Haarzellen betragt ca 1 μm

4. Diskussion

4.1 FEHLERDISKUSSION

Aus der spezifischen Aktivität von 19.4 Ci/mmol und der applizierten Dosis von 33.3 µCi/g Körpergewicht ergeben sich 0.0017 µmol DL-Leucin pro Gramm Körpergewicht; die applizierte Menge Aminosäure liegt unter 3% der des freien Leucins im Milliliter Plasma oder Perilymphe (Crifo und Crifo 1971). Das Ausmaß dieser Konzentrationszunahme bleibt im physiologischen Schwankungsbereich des Leucinspiegels. Injiziert wurde ein gleichmäßig markiertes Leucin-Razemat. Die geringe Menge des L-Isomers verschiebt somit nicht das intra- und extrazelluläre Aminosäuregleichgewicht. Eine Erhöhung der Umsatzraten von Proteinen tritt erst nach erheblich größeren Dosen auf (Roberts und Zomzely 1966). Der Einfluß des applizierten D-Isomers ist nicht zu überschauen. Die Inkorporation des ³H-L-Leucins bleibt jedenfalls ungestört, da die intraperitoneale Gabe von D-Leucin keine Wirkung auf den L-Leucin-spiegel im Plasma und im Gehirn zeigt (Lajtha und Toth 1963).

Quantitative Methoden zur Überprüfung der spezifischen Proteinmarkierung am histologischen Schnitt sind nicht verfügbar (vgl. Merei und Gallyas 1964). Die extrazelluläre Markierung über den Gefäßlumina ist vermutlich auf Albumine zurückzuführen, für deren Synthese in der Leber 30 Minuten benötigt werden (Peters 1962; McFarlane 1963). Sie könnte auch auf eine unspezifische Bindung des Vorläufers durch Glutaraldehyd hinweisen (Peters und Ashley 1967, dagegen Hodson und Marshall 1967). Jedoch liegt die kürzeste Versuchszeit mit 60 Minuten wesentlich über den 12–15 Minuten zwischen Gabe des Vorläufers und der Fixierung sowie die Konzentration des freien ³H-Leucins deutlich unter 100 µCi/g Körpergewicht; hierbei sinkt das Ausmaß der unspezifischen Bindung erheblich ab und beträgt für Glutaraldehyd wie Formalin weniger als 2% (Bergeron und Droz 1968; Monneron und Moulé 1969; dagegen Vanha-Perttula und Grimley 1970). Eine weitere Abnahme der unspezifischen Markierung, z. B. durch freie Moleküle des radioaktiven D-Isomers oder durch Nichtproteine, die nach metabolischem Abbau des ³H-Leucins tritiiert sein können, ist durch das 24stündige Auswaschen im mehrfach gewechselten Sörensen-Puffer zu erwarten, da sich der Anteil unspezifisch gebundener Substanzen stark reduziert, wenn die Spülzeit im Vergleich zur Fixierungsdauer lang ist (Peters und Ashley 1967; Vanha-Perttula und Grimley 1970).

Gegenüber der Fixierung mit Osmiumsäure bleibt der Proteingehalt von Zymogengranula nach Glutaraldehyd erhalten (Amsterdam und Schramm 1966). Demnach dürfte im vorliegenden Versuch der präparationsbedingte Verlust an markierten Proteinen (Beerstein und Tonino 1975) gegenüber dem bei anderen Fixiermitteln niedrig anzusetzen sein.

Der Einfluß der β-Selbstabsorption auf die Markierungsrelation Zellkern/Cytoplasma ist nach Kunststoffeinbettung gegenüber den Ergebnissen von Maurer und Primbsch (1964) wahrscheinlich niedriger, da bei homogener Durchdringung der Zellen mit Epon insgesamt nur die oberste, ca. 2 µm dicke Gewebsschicht zum Autoradiogramm beiträgt (Pollister 1965; Caviness und Barkley 1971).

Die verschiedenen Fixiermittel verursachen an der häutigen Schnecke unterschiedliche

Volumenänderungen (Werner, 1937) Nach Paraffineinbettung schrumpfen die Gewebe bis zu 20% (Romeis, 1968) Da autoradiographisch die Radioaktivität pro Volumeneinheit erfaßt wird und bei konventioneller Methodik unterschiedliche Volumenänderungen auftreten, verschieben sich in der Cochlea die Silberkornrelationen zwischen den einzelnen Zelltypen Im vorliegenden Versuch ist mit einer erheblich geringeren Verschiebung dieser Relationen zu rechnen, da nach Glutaraldehydfixierung die morphologische Erhaltung wesentlich besser ist (Sabatini und Mitarb., 1963) und die Schrumpfung nach Epoxydharzeinbettung nur ca 4% beträgt (Fengel und Balser, 1964)

Der durchschnittliche Meßfehler bei photometrischer Bestimmung der Silberkorndichte beträgt ±6% (s Anhang), beim visuellen Auszählen liegt er höher Trotz langer Expositionszeit der Autoradiogramme ist die Hintergrundsmarkierung mit 0,2 bis 0 4 Silberkörner pro 16 μm^2 niedrig Beim Auszählen unterblieb eine Korrektur, beim photometrischen Messen ergab sie sich durch den Abzug der Untergrundhelligkeit

Die Abgrenzung zwischen Zellkern und Cytoplasma des Striaepithels sowie zwischen Fibrozyten und Interzellularspalten des Ligamentum spirale ist lichtmikroskopisch schwierig Die Meßwerte liegen daher für ersteres zu hoch für letztere zu niedrig Bei anderen Geweben wie Böttcherzellen tympanale Belegschicht Reißnersche Membran und Zellen der Scala vestibuli, sind Anteile von Zellkernflächen in die Auswertung mit einbezogen da die Abgrenzung gegenüber dem Cytoplasma zu aufwendig ist

Bei Schnittserien kann ein Zellkern in mehreren aufeinanderfolgenden Schnitten getroffen worden sein Damit können von einem Kern mehrere unterschiedliche Durchmesser stammen Bei einem durchschnittlichen Zellkerndurchmesser von 5,6 μm und 7,4 μm betrug der kleinste noch gemessene 5,14 μm Nimmt man für Kerne der Haarzellen eine Kugelform an so sind bei einer Schnittdicke von 3 μm und einem Kugeldurchmesser von 8 μm höchstens zwei unterschiedliche Durchmesserpaare zu erhalten Dieser Fehler läßt sich nur durch die Häutchenpräparation des Cortischen Organs völlig vermeiden (Neubert, 1952)

4 2 PROTEINSYNTHESE, MORPHOLOGISCHE BEOBACHTUNGEN

Wegen der komplizierten Struktur des Innenohres ist bisher der morphologische Erhaltungsgrad der Cochlea nach üblicher Entkalkung und Paraffineinbettung des Schläfenbeins erfahrungsgemäß unbefriedigend Er ist nach der hier verwendeten Kunststoffeinbettung bis in den submikroskopischen Bereich leicht zu reproduzieren

Biochemische Grundlage der autoradiographischen Reaktion über den Zellen nach ^3H-Leucin ist der Einbau der Aminosäure in Proteine Die zelluläre Proteinsynthese erfolgt unter Codierung durch die *m*-RNA und durch deren Wechselwirkung mit der Aminoacyl *t*-RNA an den Polysomen (Biochemische Übersichten Hendler, 1968, Spirin und Garviola, 1969, Stewart und Letham, 1973) Autoradiographisch zeigt die früheste Markierung über Zellen nach Applikation radioaktiver Aminosäuren den Ort der Proteinsynthese an, während eine spätere Markierung über anfänglich wenig oder keine Radioaktivität enthaltenden Zellstrukturen auf eine langsame Syntheserate zurückzuführen ist oder darauf, daß Proteine vom Syntheseort diese Zellstrukturen erreicht haben

Der ribosomalen Eiweißsynthese entspricht bei den Zellen des Innenohres die Markierung über dem Cytoplasma Der Syntheseort ist jedoch nur bei Versuchszeiten von 2 bis 10 Minuten festzustellen, da bei längeren Zeitspannen der Markierungsanteil anderer Zellstrukturen im Vergleich mit dem des endoplasmatischen Reticulums deutlich zunimmt (Droz, 1967, Young und Droz, 1968) Offensichtlich bleibt jedoch wie bei Nervenzellen der Spinalganglien und bei Rezeptoren der

Retina auch bei Ganglienzellen der Cochlea eine hohe Silberkorndichte uber dem endoplasmatischen Reticulum fur lange Zeit bestehen, die zum Teil der Inkorporation markierter Aminosauren in Strukturproteine der Ribosomen entsprechen durfte Der kleine Anteil zum Golgi Komplex wandernder Proteine laßt sich bei den Nervenzellen im vorliegenden Versuch mit der kurzesten Markierungszeit von 1 Stunde nicht erfassen (Droz, 1967, Young und Droz, 1968) Lediglich weiter entfernte Zellanteile, wie Axone und Mitochondrien, *lassen durch zeitabhangige Zunahme* der Silberkorndichte eine intrazellulare Verlagerung von Proteinen erkennen

Ein den Zellen der Cochlea gleiches Verteilungsmuster der Markierung ist fur eine große *Anzahl anderer Gewebe* beschrieben (Ross und Benditt 1965, Tixier-Vidal und Picart, 1967, Young und Droz, 1968, Bergeron und Droz, 1969, Nakagami und Mitarb , 1971, Meyrick und Reid 1975, bei Ganglienzellen Droz und Leblond 1963, Droz, 1967, Droz und Koenig, 1969) Demnach entspricht der Verlauf der Proteinsynthese in den Zellen der Cochlea dem in anderen Zellen

Nach Caspersson (1950) ist die Große der Eiweißsynthese innerhalb der Zellen von der Menge der RNA abhangig In der Ultrastruktur weisen Ganglienzellen und *Striaepithelien pro Volumeneinheit im Cytoplasma einen* wesentlich hoheren Gehalt an Ergastoplasma auf als Sulcusepithelien und in inneren Haarzellen finden sich relativ mehr cytoplasmatische Organellen als *in außeren Haarzellen* (Spoendlin 1966 Iurato 1967) Dem entspricht cytophotometrisch der relative RNA Gehalt von 60% fur Striaepithelien und von 15% fur Sulcusepithelien bezogen auf 100% fur Nervenzellen des Ganglion *spirale* cochleae (Kraus, 1970) Mit dem RNA Gehalt und der Ultrastruktur des membranosen Labyrinthes stimmen die starken Schwankungen der Silberkorndichte mit einem Maximum bei *Ganglienzellen* und einem *Minimum bei Sulcusepithelien* uberein Zudem uberwiegen in Ganglienzellen und Striaepithelien das rauhe *und geordnete endoplasmatische Reticulum* (Iurato, 1967, Rosenbluth, 1967), dagegen in Sulcusepithelien und außeren Haarzellen die freien Polysomen (Spoendlin 1966) Anscheinend besitzen membrangebundene Ribosomen eine hohere Inkorporationsrate und synthetisieren zur Sekretion bestimmte oder innerhalb der Zellen wandernde Proteine (Siekevitz und Palade, 1960, Ross und Benditt, 1965, Droz, 1967, Young und Droz, 1968, Redmann, 1969 Sherr und Uhr, 1970, Andrews und Tata 1971, Ikehara und Pitot, 1973, Black und Webster, 1973, *Harwood und Mitarb , 1975*), wahrend Proteine fur den endogenen Bereich auch an den freien Polysomen entstehen (Redmann 1969, Ganoza und Williams 1969)

Im vorliegenden Versuch zeigen Mitochondrien eine *Stunde* nach *Applikation* der markierten Aminosaure vereinzelt eine autoradiographische Reaktion die der mitochondrialen Proteinsynthese zuzuordnen ist (Beattie und Mitarb , 1967, Bergeron und Droz, 1969, Lizardi und Luck, 1972) In der Großenordnung ist diese jedoch innerhalb der Zellen gegenuber der des *endoplasmatischen* Reticulums zu vernachlassigen, da an Mitochondrien wahrscheinlich nicht mehr als 12 verschiedene Polypeptide, d h Fraktionen von Apoenzymen, synthetisiert werden und der uberwiegende *Anteil mitochondrialer Proteine cytoplasmatischen Ursprungs ist* (Ubersicht Schatz und Mason, 1974) Die mit der Versuchszeit zunehmenden Mitochondrienmarkierung erklart sich demnach am ehesten wie *auch in Sinneszellen der Retina (Young* und Droz 1968) aus dem Einbau cytoplasmatischer markierter Proteine

Auffallend ist die geringe Markierung des Hensenkorpers in den außeren Haarzellen *der in seiner Ultrastruktur (Spoendlin* 1966) dem Nebenkern entspricht Histochemisch ist er dem endoplasmatischen Reticulum zuzuordnen (Kuttner, 1975) Er wird als morphologisches Aquivalent einer intensiven Proteinsynthese *gedeutet (Ubersicht Molbert*, 1968) Die vorliegenden Befunde sprechen gegen diese Ansicht Ein Grund für die relativ nied

rige Markierung des Lamellarkörpers konnte auch eine hohe *Syntheserate* des *subplasmalemmalen* Zisternensystem sein

Bei erwachsenen Tieren läßt sich lichtmikroskopisch ein Proteinumsatz in den Phalangealzellen und in der Basilarmembran kaum erfassen (Koburg und *Plester, 1962a) Dem*gegenüber zeigen im vorliegenden Versuch junge Meerschweinchen hier eine deutliche Aminosäureinkorporation Insbesondere der stärkere Einbau über dem Bereich fibrillärer *Proteine* der *Pfeilerzellen* bestätigt die bei Ratten beobachtete Vermehrung der Tonofibrillen bis zur Adoleszenz (Iurato und Fedrizzi, 1961) Die Dichte der Basilarmembran nimmt bis zur Geschlechtsreife stark ab, während *des Alterns wird sie nur gering reduziert* (Kraus, 1970) Ob hierbei Änderungen in der Eiweißkonzentration eintreten, ist bisher nicht untersucht; zumindest für den vergleichbar bradytrophen Glaskörper des Auges wird im Alter *eine verminderte* Proteinkonzentration beschrieben (Bembridge und Pirie, 1951 Burger und Friedel, 1958) Offensichtlich geht nach vorliegenden Ergebnissen die altersabhängige Abnahme der Basilarmembrandichte mit einer Reduzierung des Eiweißstoffwechsels parallel

Nach erheblicher Schallbelastung wird für die Blutgefäße der Stria vascularis eine Störung der Mikrocirculation beschrieben (Kellerhals 1972) Da im vorliegenden Versuch die dichte Packung von Erythrozyten nur in vereinzelten Gefäßlumina bei beschallten Tieren und nicht häufiger *als bei der Kontrollgruppe* gefunden wird, scheidet die Schallbelastung als Ursache dieses Phänomens hier aus Vielmehr ist mit einer praefinalen Stase im Endstromgebiet als Folge der Äthernarkose zu rechnen die vor der Dekapitation erfolgte und deren Tiefe am Reflexverhalten der Versuchstiere nur unzureichend abgeschätzt werden *konnte*

Vom Versuchsbeginn an sind alle Zellkerne der Innenohrgewebe stark markiert Diese Inkorporation wird bei vielen Geweben auch nach Versuchszeiten von 2–5 Minuten gefunden Aus der Kürze dieses Zeitintervalls und *aus dem konstanten Verhältniswert zwischen* Karyo- und Cytoplasmamarkierung wird auf eine nukleare Proteinsynthese geschlossen (Leblond und Amano, 1962, Monesi, 1964 Schultze und Maurer, 1967, Droz, 1967, Ha*milton und Mitarb* , *1968*, Schultze, *1969)* Dafür sprechen ebenso biochemische und autoradiographische Untersuchungen an isolierten Zellkernen, die weiterhin die Fähigkeit, Aminosäuren in Kernproteine einzubauen, beibe*halten (Übersicht* *Allfrey, 1970) Von diesen* relativ einheitlichen Ergebnissen bei Thymus und Milzzellen weichen jedoch die biochemischen Befunde für Nieren , Leber- und Nervenzellen ab (Übersicht Kuehl, 1974) so daß *der allgemeine* Schluß *auf eine* nukleare Eiweißsynthese in jedem Gewebe nicht gerechtfertigt erscheint, zumal lymphozytäre Zellen mit einem Kernvolumen, das ca 60% der Zelle ausmacht eine Sonderstellung einneh*men Im Gegenteil sprechen eine Reihe* von Befunden dafür, daß Kernproteine im Cytoplasma synthetisiert werden und dann in den Zellkern wandern Dies ist autoradiographisch, cytochemisch oder durch Transplantation von Zellkernen bei Amöben (Goldstein 1958, Byers und Mitarb , 1963, Prescott und Bender, 1963), bei Fibroblasten (Zetterberg, *1966a b)*, bei HeLa-Zellen (Speer und Zimmermann, 1968, Stein und Baserga, 1971), bei Nervenzellen (Larra, 1970) sowie biochemisch für Histone (Stellwagen und Cole, 1969), für ribosomale Proteine (Heady und McConkey, *1970, Craig und Perry, 1971) und für Proteine* des Nucleolus (Speer und Zimmermann, 1968, Kawashima und Mitarb , 1971) belegt

Eine nukleare Eiweißsynthese ist demnach für lymphozytäre Zellen anzunehmen, für andere Gewebe ist diese *Frage nicht entschie*den Zumindest für den überwiegenden Anteil der Kernproteine wird deren cytoplasmatische Genese angenommen (Goldstein, 1970, Kuehl, 1974) Aufgrund der vorliegenden Autoradiogramme ist daher kein sicherer Rückschluß auf eine nukleäre Proteinsynthese der Innenohrgewebe möglich

Bei Zellkernproteinen werden zwei Fraktionen unterschieden die mit verdünnten Sauren extrahierbaren Histone und die dann noch DNA-gebunden bleibenden Nichthiston-Proteine Da Leucin in alle Proteine eingebaut wird, kann in vivo ein spezifischer Eiweißkorper allein nicht markiert werden Lediglich die Lokalisation der Silberkorner über dem Chromatin erlaubt einige Ruckschlusse das Heterochromatin und weite Bereiche des Euchromatins zeigen bei Ganglien- und Schwannschen Zellen der Cochlea nur gelegentlich eine autoradiographische Reaktion Demgegenüber zeigen die grenznahen Bereiche von Eu und Heterochromatin, die „Perichromatinregion", eine stärkere Markierung, die auch für andere Zellkerne beschrieben ist (Bouteille, 1972) und hier einen größeren Proteinumsatz vermuten laßt Der Umsatz von Histonen ist eng mit dem DNA-Stoffwechsel korreliert (Byvoet, 1966) und betragt bei Zellen des Hirngewebes 0,6–1,3% in 24 Stunden (Piha und Mitarb , 1966) Fur die hautigen Gewebe des Innenohres ist ein größerer Umsatz nicht zu erwarten, da eine Erneuerung nervoser Elemente nicht erfolgt und autoradiographisch an erwachsenen Tieren praktisch kein Einbau von ^{3}H-Thymidin gefunden wird (Koburg, 1961, Watanuki und Mitarb , 1968) Ein niedriger Ruhestoffwechsel der DNA, z B zur Strahlenreparatur, ist zu vermuten, jedoch autoradiographisch nicht zu erfassen Es ist daher anzunehmen, daß die Markierung uber den Zellkernen des Innenohres kaum dem Umsatz von Histonen zuzurechnen ist

Gegenuber Histonen wird in ruhenden Zellen eine hohere Markierungs- und Syntheserate der Nichthiston Proteine angegeben (Allfrey und Mitarb 1955 Busch 1965) Die Markierung uber den Zellkernen der Gewebe der Cochlea ist daher am ehesten auf histonfreie Proteine zuruckzufuhren denen bei der Genregulation eine entscheidende Bedeutung zugeschrieben wird (Ubersicht Olson und Busch, 1974)

Der Ubergangsbereich beider Chromatinformen, die Perichromatinregion ist besonders aktiv in der Synthese extranuklearer RNA (Fakan und Bernhard, 1971, Petrov und Bernhard, 1971) Die starke Markierung dieser Kernregion bei Ganglien- und Schwannschen Zellen nach ^{3}H-Leucin laßt vermuten, daß hier Proteine an der RNA-Synthese beteiligt sind, sei es als komplementierender Anteil von Ribonucleoproteinen oder Enzymen

Im Nucleolus werden ribosomale RNA-Vorlaufer mit ribosomalen Proteinen kombiniert, die im Cytoplasma synthetisiert werden (Heady und McConkey, 1970, Craig und Perry, 1971, Ubersicht Stewart und Letham, 1973) Innerhalb von Nervenzellen liegt die Eiweißkonzentration des Nucleolus uber der des restlichen Zellkerns und der des Cytoplasmas (Nurnberger und Mitarb , 1952, Hydén und Larsson, 1956), so daß die starke Markierung uber dem Kernkorperchen als Hinweis auf eine sehr rasche Erneuerung dieser Proteine gedeutet werden muß

4 3 KINETIK

Im vorliegenden Versuch wurde die Silberkorndichte uber verschiedenen Geweben des Innenohres 1 bis 24 Stunden nach intraperitonealer Injektion von ^{3}H-DL-Leucin bestimmt und im zeitlichen Verlauf ein Markierungsmaximum nach 2 bis 3 Stunden sowie im Langzeitversuch nach Abnahme der Silberkorndichte ein erneuter Anstieg bis 24 Stunden festgestellt

Es fragt sich, ob die Art der Applikation fur den Verlauf der Silberkorndichte verantwortlich ist Fur die Gewebe ist ein Verlauf der Markierungsdichte zu erwarten, der von der Konzentrationsverteilung der radioaktiven Aminosaure wahrend der Versuchszeit abhangt Durch intravenose Injektion wird zu Versuchsbeginn eine hohe Konzentration erreicht, die relativ rasch wieder abfallt (Droz, 1967) Diesem kurzen, radioaktiven Signal entspricht autoradiographisch und biochemisch eine schnelle Abnahme der Aktivitat nach einem Markierungsmaximum Nach intraperitonealer Injektion verlauft die Konzentra

tionskurve des Tracers im Blut erheblich flacher; sie fällt gegenüber 2 Minuten nach i. v.-Applikation erst nach 4 Stunden auf 10% des Maximums kurz nach der Injektion ab (Ross und Benditt, 1965). Das radioaktive Signal ist demnach deutlich länger, so daß autoradiographisch eine flach verlaufende, im Markierungsmaximum nicht so stark ausgeprägte Kurve wie nach i. v.-Applikation der radioaktiven Substanz zu erwarten ist. Offensichtlich sind jedoch diese zeitlichen Konzentrationsunterschiede zwischen beiden Injektionswegen bei Markierungszeiten von 1 Stunde und länger wie im vorliegenden Versuch nicht von wesentlicher Bedeutung für den Verlauf der Silberkorndichte, da auch nach intraperitonealer Injektion für Leber und Niere eine rasche Abnahme der autoradiographischen Markierungsdichte (Romen und Hempel 1975) sowie der sepzifischen Aktivität der Organproteine (Lim und Agranoff, 1969) gefunden wird. Vermutlich überwiegt bei beiden Applikationsformen der Anteil markierter Proteine mit raschem Umsatz, deren spezifische Aktivität daher schnell abfällt, während Proteine mit einem langsamen Umsatz erst durch ein erheblich länger andauerndes Angebot radioaktiver Substanzen gleichmäßig markiert und bei kinetischen Untersuchungen ausreichend erfaßt werden (Garlick und Marshall 1972, Lajtha und Mitarb., 1976, Garlick und Mitarb., 1976). Es ist daher anzunehmen, daß im vorliegenden Versuch der gewählte Injektionsweg keinen unmittelbaren Einfluß auf den zeitlichen Verlauf der Silberkorndichte hat.

Gegenüber der höchsten Silberkorndichte über den Geweben des Intestinuums 1 Stunde nach Aminosäureinjektion (Romen und Hempel, 1975) ist für Zellen des Gehirns sowohl nach intravenöser wie intraperitonealer Applikation das autoradiographische und biochemische Markierungsmaximum mit 1,5 bis 4 Stunden deutlich verzögert (Droz und Leblond 1963, Jakoubek und Mitarb., 1968, Bloomstrand und Hamberger, 1969, Lim und Agranoff 1969, Semiginovsky und Jakoubek, 1971) und das Abfallen der Markierungsdichte gegenüber anderen Organen erheblich langsamer (Lim und Agranoff, 1969, Pakkenberg und Fog, 1973). Der zeitliche Verlauf der Silberkorndichte über den Zellen der Cochlea im vorliegenden Kurz- und Langzeitversuch entspricht somit bis zur 4. bzw. 8. Stunde den Ergebnissen von Nervenzellen des Gehirns.

Abweichend von bisherigen Mitteilungen in der Literatur zeigt sich im Langzeitversuch ein Wiederanstieg der Silberkorndichte bis zum 24-Stunden-Wert, wobei für einzelne Zellarten Werte des 1. Maximums überschritten werden. Als Ursache dafür kommen der Tracer, eine organspezifische Kinetik oder allgemeine Änderungen im Tierkörper in Frage. Trotz Verwendung des ³H-Leucin-Razemates ist im Kurvenverlauf nach einem Hochstwert lediglich ein kontinuierliches Abfallen der Markierungsdichte wie bei autoradiographischen Untersuchungen von Ganglienzellen des Gehirns (Droz und Leblond, 1963) zu erwarten. In Nervenzellen wird D-Leucin weder in Proteine eingebaut noch metabolisiert (Lajtha und Toth, 1962, Clouet und Neidle, 1970), so daß auch für das Innenohr eine Inkorporation des D-Isomers, eventuell verzögert gegenüber dem L-Isomer, als möglicher Grund des Wiederanstieges der Markierungsdichte nicht wahrscheinlich ist. Auch scheidet eine den Perilymphräumen eigene Kinetik ursächlich aus, da diese zumindest für Natrium- und Chlorionen mit einer einfachen e-Funktion 1. Ordnung beschrieben werden kann (Portmann und Mitarb., 1960, Jung, 1975).

Da gelegentlich bei *Langzeituntersuchungen* für Hirngewebe nach alleiniger Verwendung des Isomers ³H-L-Leucin *in vivo* ebenso eine erneute Zunahme proteingebundener und freier Radioaktivität gefunden wird (Bloomstrand und Hamberger, 1969, Pakkenberg und Fog, 1973), dürfte die Zunahme wie im vorliegenden Langzeitversuch auf Vorgänge außerhalb des untersuchten Organs zurückzuführen sein. Hierbei ist besonders der zwischen Organen stattfindende Austausch von Aminosäuren zu berücksichtigen, der z. B. nach

intraperitonealer Traceriniektion wahrend der Abnahme der spezifischen Aktivitat von Leberproteinen durch Reutilisation markierter Aminosauren eine Zunahme der Radioaktivitat in Muskelproteinen bewirkt (Waterlow und Stephen, 1966) Unter Nahrungsentzug werden von Organen des Intestinuums im unterschiedlichen Ausmaß Aminosauren vermehrt an das Blut abgegeben, um vermutlich den Bedarf anderer, lebenswichtigerer Organe zu decken (vgl Munro und Allison, 1964, Gan und Jeffay, 1967, Millward, 1970) Fur Hirngewebe wird bei nicht ausreichender Diat eine starkere Inkorporation von radioaktivem Leucin bei gegenuber Kontrollen erhohtem Blutspiegel gefunden (Roberts und Morelos, 1965) Im vorliegenden Versuch befinden sich die Tiere zum Zeitpunkt der Aminosaureinjektion *nach vorangegangenem Futtern in einer noch* annahernd ausgeglichenen Stoffwechsellage (Elwyn 1970), so daß anfanglich ein typischer Verlauf der Markierungskurve erreicht wird Mit zunehmender Fastendauer wahrend des Versuches ist durch den Abbau von Proteinen innerer Organe (Gan und Jeffay, 1967) ein erneuter Anstieg an radioaktiver Aminosaure in der Blutbahn zu erwarten, wodurch eine starkere Proteinmarkierung des Gehirns und im vorliegenden Fall des Innenohres zustande kommt Der erneute Anstieg der Silberkorndichte nach 24 Stunden ist demnach durch die Kinetik und Reutilisation der Aminosaure unter der sich wahrend des 36-stundigen Hungerns andernden Stoffwechsellage zu erklaren Ein Vergleich mit anderen Organen zur Stutzung dieser Annahme wurde nicht durchgefuhrt

Absolute Unterschiede in der Silberkorndichte der entsprechenden Gewebe beider Versuchsreihen ergeben sich aus methodischen Abweichungen Im 24 Stunden-Versuch erfolgt die Markierung in der Erholungsphase nach Beschallung mit 85 db Rauschen und im Wachzustand, jedoch im Kurzversuch wahrend des Schallreizes und in Narkose Insbesondere ist unter der Wirkung von Chlorpromazin und Pentobarbital im Kurzversuch mit einer erheblich reduzierten Inkorporation von ^{3}H-Leucin zu rechnen (Gaitonde und Richter, 1956, Peterson und Mitarb , 1972) Ferner ist beim Vergleich eine unterschiedlich lange Expositionszeit der Autoradiogramme zu berucksichtigen

Gegenuber dem gut vascularisierten Gewebe des Ganglienlagers des Modiolus und der Stria vascularis wird das Cortische Organ vorwiegend uber die Cortilymphe metabolisch versorgt, wodurch das Angebot von ^{3}H-Leucin verzogert wird (Meyer zum Gottesberge und Plester, 1961) Bezogen auf die Silberkorndichte uber den Nervenzellen des Ganglion spirale cochleae ergibt sich jedoch fur Haar- und Stutzzellen im vorliegenden Versuch zu allen Zeiten eine weitgehend gleiche prozentuale Relation Hieraus ist zu schließen, *daß eine Stunde nach Applikation die Testsubstanz* im Innenohr gleichmaßig verteilt ist und Verzögerungen durch den Stofftransport zum Cortischen Organ fur dessen Silberkorndichte zu diesem Zeitpunkt keine Bedeutung mehr besitzen

4 4 BLUT-PERILYMPHSCHRANKE

Hier wie in fruheren Versuchen (Koburg und Plester, 1962*a*, Richrath und Kraus, 1972) wird fur die Cochlea im Vergleich zu anderen Geweben eine relativ geringe Markierung gefunden Hohe Dosen von ^{3}H-Leucin sowie lange Expositionszeiten sind erforderlich Demnach besteht fur Leucin nicht nur eine partielle Bluthirnschranke (Lajtha und Toth, 1961, Schultze und Mitarb , 1972), sondern auch eine partielle zwischen Blut und Lymphen des Innenohres Vergleichbare Ergebnisse finden sich bei ^{3}H-Cytidin (Kraus und Mitarb , 1975) Hieraus und aus der Transportbarriere des Gehirns und der Cochlea gegenuber γ-Aminobuttersäure (Richrath und Mitarb , 1974) ist zu vermuten, daß beide Schranken sich auch gegenüber anderen Metaboliten identisch verhalten und damit der Blut-Lymphschranke fur das Innenohr die gleiche Bedeutung in der Kontrolle des Stoff-

wechsels zukommt wie der Bluthirnschranke für das Gehirn (Lajtha, 1968)

4.5. AXOPLASMATISCHE PROTEIN-WANDERUNG

Im vorliegenden Versuch folgt bei Ganglienzellen der höchsten Markierung des Cytoplasmas 2 bis 3 Stunden nach Tracerapplikation eine besonders starke Abnahme der Silberkorndichte zum 4- bzw 8-Stunden-Wert Nach gleichem Zeitintervall wird für Nervenzellen des Gehirns biochemisch die Abgabe einer Proteinfraktion (Bloomstrand und Hamberger, 1969, Rose und Sinha, 1974) und autoradiographisch eine Neuverteilung von Proteinen innerhalb der Ganglienzellen mit starker Markierung über dem Ursprungskegel der Axone (Droz und Leblond 1963) beschrieben Ebenso zeigt sich im Nervus opticus eine zentripedale Wanderung markierter Proteine nach intraoculärer Tracerinjektion (Karlsson und Sjostrand, 1971, Grafstein und Mitarb., 1972) Demnach dürfte die Abnahme der Silberkorndichte uber den Ganglienzellen des Innenohres zum Teil auf eine Abgabe von Proteinen an die Axone zuruckzufuhren sein Fur den Nervus acusticus ist die axonale Wanderung der in den Ganglienzellen synthetisierten Proteine bisher nur indirekt nachgewiesen Mit zunehmender Versuchszeit steigt die Markierungsdichte uber zentripedalem und fugalem Axonenbereich in Relation zur Silberkorndichte uber dem Pericaryon kontinuierlich an, wobei fur beide Nervenabschnitte wegen des identischen Kurvenanstieges eine gleiche Wandergeschwindigkeit der Proteine anzunehmen ist (Haubrich und Koburg 1967) Versuchsanordnung und Auswertung erlauben keine Ruckschlusse auf unterschiedlich schnell wandernde Eiweißfraktionen, nach den Befunden an anderen Hirnnerven (Ubersicht Lubinska 1975) ist dies jedoch auch fur den Nervus acusticus zu erwarten Der uberwiegende Anteil schnell wandernder Proteine besteht aus Mikrosomen mit einer großen Anzahl verschiedener Polypeptide (Willard und Mitarb., 1974) und durfte der Funktionserhaltung von Axonen und Synapsen dienen (Cancalon und Beidler, 1975) Als deren Strukturprotein ist die langsam wandernde Eiweißfraktion anzusehen (Frizell und Sjostrand 1974*a*), die sich zudem nur aus einer geringen Anzahl von Polypeptiden zusammensetzt (Hoffman und Lasek 1975)

Die Autoradiogramme vorliegender Versuchsreihen zeigen eine relativ schwache Markierung des Nervus acusticus wobei lichtmikroskopisch keine ausreichend sichere Zuordnung der einzelnen Silberkorner zu Axonen oder deren Myelinmantel gewährleistet ist Auf eine Auswertung zur axonalen Wanderung von Proteinen wurde verzichtet, zumal nach systematischer Tracerapplikation ebenfalls eine Markierung des Hullgewebes erfolgt

Fur den weiteren Kurvenverlauf im Langzeitversuch ist ferner der zellulipedale Transport von Proteinen zu berucksichtigen, der moglicherweise als Ruckkopplung zwischen spezifischer und metabolischer Zellaktivität dient Die Menge retrograd wandernder Proteine erreicht innerhalb von 21–24 Stunden ca 50–70% der Großenordnung des schnellen zellulifugalen Transportes (Bray und Mitarb., 1971, *Lubińska und Niemierko, 1971, Fonnum* und Mitarb., 1973) mit einer Wandergeschwindigkeit von 60–140 mm/24 Stunden (Kristensson und Mitarb., 1971, Edström und Hanson, 1973, Frizell und Sjöstrand, 1974*b*) Da beim Meerschweinchen die Entfernung des Ganglion spirale cochleae von den Haarzellen ca 0 6 mm (Fernandez, 1952) und vom Nucleus cochlearis im Hirnstamm ca 12 mm betragt, dürfte im vorliegenden Versuch der retrograde Transport markierter Proteine relativ früh einsetzen Fur die erneute Zunahme der Radioaktivitat in den Nervenzellen des Ganglion spirale cochleae nach 24 Stunden muß jedoch der Reflux und die Reutilisation dieser Proteine von untergeordneter Bedeutung sein, da mehr als die Halfte der die Zelle innerhalb von 24 Stunden verlassenden Proteine der langsam wandernden Fraktion zuzurechnen ist und deren Menge an Radioaktivitat ca. zehnmal

intraperitonealer Traceriniektion während der Abnahme der spezifischen Aktivität von Leberproteinen durch Reutilisation markierter Aminosäuren eine Zunahme der Radioaktivität in Muskelproteinen bewirkt (Waterlow und Stephen, 1966) Unter Nahrungsentzug werden von Organen des Intestinuums im unterschiedlichen Ausmaß Aminosäuren vermehrt an das Blut abgegeben, um vermutlich den Bedarf anderer, lebenswichtigerer Organe zu decken (vgl Munro und Allison, 1964, Gan und Jeffay, 1967, Millward, 1970) Für Hirngewebe wird bei nicht ausreichender Diät eine stärkere Inkorporation von radioaktivem Leucin bei gegenüber Kontrollen erhöhtem Blutspiegel gefunden (Roberts und Morelos, 1965) Im vorliegenden Versuch befinden sich die Tiere zum Zeitpunkt der Aminosäureinjektion nach vorangegangenem Füttern in einer noch annähernd ausgeglichenen Stoffwechsellage (Elwyn, 1970), so daß anfänglich ein typischer Verlauf der Markierungskurve erreicht wird Mit zunehmender Fastendauer während des Versuches ist durch den Abbau von Proteinen innerer Organe (Gan und Jeffay, 1967) ein erneuter Anstieg an radioaktiver Aminosäure in der Blutbahn zu erwarten, wodurch eine stärkere Proteinmarkierung des Gehirns und im vorliegenden Fall des Innenohres zustande kommt Der erneute Anstieg der Silberkorndichte nach 24 Stunden ist demnach durch die Kinetik und Reutilisation der Aminosäure unter der sich während des 36 stündigen Hungerns ändernden Stoffwechsellage zu erklären Ein Vergleich mit anderen Organen zur Stützung dieser Annahme wurde nicht durchgeführt

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1975) Entsprechende Befunde werden bei der Maus für das Leucinrazemat angegeben (Schultze und Mitarb., 1960). Die Ursache ist vermutlich der D-Aminosäureoxydase zuzuschreiben. Für die Enzymaktivität werden neben den Unterschieden zwischen einzelnen Tierespezies und zwischen Organen spezifische Unterschiede bei den einzelnen D-Aminosäuren beschrieben (Krebs, 1935; Bender und Krebs, 1950). *In vitro* besitzt die Enzymreaktion mit D-Leucin die gleiche Größenordnung wie mit DL-Prolin (Klein und Handler, 1941). Bei Meerschweinchen enthalten die Leukozyten eine D-Aminosäureoxydase, deren Aktivität nur wenig unter der von Nierengewebe liegt (Cline und Lehrer, 1969).

Im vorliegenden Versuch mit dem ^{3}H-Leucinrazemat ist demnach die molekulare Zuordnung der Radioaktivität im Pool der Innenohrzellen nur schwer abzuschätzen. Neben ^{3}H-L-Leucin ist mit einer Markierung anderer Aminosäuren und von Metaboliten zu rechnen, wobei sich die verschiedenen Moleküle zusätzlich durch ihre Kinetik in der Perilymphe unterscheiden können (Jung, 1975). Da sich im Gehirn 14 Tage nach intraperitonealer Injektion von ^{3}H-Leucin 85% der an Proteine gebundenen Aktivität in der gleichen Aminosäure wiederfinden (Lim und Agranoff, 1969), ist auch bei den Zellen der Cochlea eine rasche Aufnahme von Leucin in den endogenen Pool und eine effektive Inkorporation in Proteine zu erwarten. Auch ist gegenüber der Markierung anderer Aminosäuren der Anteil tritiierter Stoffwechselprodukte nach parenteraler Applikation nicht sehr hoch (Schotman und Mitarb., 1974). Über den Markierungsanteil durch ^{3}H-D-Leucin und seiner Abbauprodukte kann im vorliegenden Versuch nichts ausgesagt werden, der größte Anteil dürfte bei der Präparation entfernt worden sein. Eine D-Aminosäureoxydase wurde im Innenohr bisher nicht beschrieben.

Genaue Daten über die Durchblutung, die Kinetik der spezifischen Aktivität von ^{3}H-Leucin in den Lymphen und über die Zusammensetzung des intrazellulären Aminosäurepools sind für die Cochlea nicht verfügbar. Ob der freie Aminosäurepool in den Zellen tatsächlich den Vorläufer für Proteine darstellt, ist bisher nicht entschieden (Goldberg und Dice, 1974). Die Zellen des Cortischen Organs könnten z. B. wegen des interzellulären Flüssigkeitsraumes bei der Proteinsynthese extrazelluläre Aminosäuren den intrazellulären vorziehen (Hider und Mitarb., 1969; dagegen Fern und Garlick, 1974). Ebenso ist für das Innenohr die Größe der Reutilisation markierter Aminosäuren aus dem Abbau von Proteinen unbekannt. Hieraus stammen ca. 50–80% der freien Aminosäuren des intrazellulären Pools bei Leberzellen, dieser Anteil variiert in den verschiedenen Geweben und ändert sich mit dem hormonellen und Ernährungsstatus (Gan und Jeffay, 1967).

4.7 ZUNAHME DER SILBERKORNDICHTE NACH BESCHALLUNG

Während der akustischen Reizung wird im Kurzversuch anscheinend vom beschallten Innenohr mehr ^{3}H-Leucin eingebaut als von der Gegenseite, wobei der zeitliche Verlauf im Markierungsverhalten für Kontrolle und Beschallung weitgehend identisch ist. Das Maximum der Silberkorndichte ist gegenüber der Kontrollgruppe des Langzeitversuches für beide Seiten schon nach 2 Stunden erreicht. Durch die Fixierung des Schalleiters am Schädelknochen und bei einer Rauschintensität von 85 db ist über die Knochenleitung auch mit einer Reizung der Gegenseite zu rechnen, die der Größenordnung nach nur ca. 10 db unter der des beschallten Ohres liegt und wahrscheinlich die geringen Seitendifferenzen im Kurzzeitversuch erklärt. Auch im zeitlichen Markierungsverhalten zeigt die beschallte Tiergruppe des Langzeitversuches deutliche Abweichungen von der Kontrollgruppe trotz Überlagerung durch die sich während der Versuchszeit ändernde Stoffwechsellage. In der Erholungsphase nach Beschallung ist neben einer stärkeren Inkorporation auch

eine Vorverlagerung der Markierungsmaxima und -minima gegenuber Kontrollen festzustellen Bei den Haarzellen und deren Zellkernen sind diese Unterschiede nicht so deutlich

Nach Reizung durch Licht wird eine Anderung der Poolzusammensetzung im Rattenhirn gefunden (Rose, 1972) Demnach konnte das gegenuber Kontrollen abweichende Inkorporationsverhalten der Innenohrzellen durch Beschallung Anderungen in der spezifischen Aktivitat des Pools und in der Kinetik von ^{3}H-Leucin durch erhohte Zellpermeabilitat oder erhohte Blutzirkulation reflektieren Zumindest fur die Perilymphe hat erst eine Schallintensitat ab 100 db Einfluß auf deren Halbwertszeit, da sie verlangert wird (Schnieder, 1974), ist eher mit einem verminderten Angebot an Aminosauren zu rechnen Unter gleichzeitiger Berucksichtigung der Poolverhaltnisse laßt sich im Gehirn eine Anderung des Proteinstoffwechsels nach massiven, afferenten Reizen nachweisen (Ubersicht Lajtha und Marks, 1971), die Wirkung physiologischer Reizstarken ist bisher nicht zu erfassen So wird in der Rattenretina nach Lichtexposition gegenuber der Netzhaut im Dunklen aufgezogener Tiere eine erhohte Inkorporation nur in vereinzelten Proteinfraktionen gefunden (Richardson und Rose, 1973)

Zum Nachweis eines Stoffwechseleffektes muß der Intensitatsunterschied zwischen Beschallung und Kontrollen offensichtlich eine bestimmte Großenordnung uberschreiten Ebenso wie die Seitenunterschiede im Kurzversuch statistisch nicht zu sichern sind, lassen sich bei Belastung durch normalen Umweltlarm autoradiographisch kaum Stoffwechselunterschiede zwischen Innenohren von gesunden Ratten und von Versuchstieren mit Otitis media feststellen (Koburg und Plester, 1962*b*) obwohl eine Dampfung des Gerauschpegels anzunehmen ist Das Ausmaß dieser Dampfung unterliegt jedoch Schwankungen zwischen 0 bis 60 db Demgegenuber besteht im Langzeitversuch ein definierter Intensitatsunterschied von 50 db und im Beschallungsversuch von Koburg und Hempel (1965) mit 90 db eine Reizstarke, die den Umweltlarm deutlich ubersteigt In beiden Versuchsanordnungen laßt sich eine erhohte Aminosaureinkorporation feststellen

Fur Innenohrzellen wird nach starker Schallbelastung eine Abnahme und nach kurzer Belastung eine Zunahme der Proteinkonzentration beschrieben (Hamberger und Hyden, 1949, Vinnikow und Titova, 1963) Demnach durfte der Abnahme der Silberkorndichte nach starker Schallbelastung (Anichin, 1970) am ehesten eine Depression der Proteinsynthese entsprechen In Anichin's Versuchen wird die Beschallungsdauer variiert, wahrend die Markierungszeit von 6 Stunden, nach denen ^{35}S-Methionin die starkste Inkorporation in Proteine der hautigen Schnecke erreicht hat, beibehalten wird Unter der Schallbelastung mit 100 db dürfte jedoch die Kinetik noch starker beeinflußt werden als im vorliegenden Langzeitversuch nach 85 db Ebenso wie ein vermindertes Aminosaureangebot wegen der verlangerten Halbwertszeit der Perilymphe (Schnieder, 1974) zu berucksichtigen ist, konnte unter der Beschallung das Maximum der Proteinmarkierung erheblich fruher erreicht sein, so daß bei festgehaltener Markierungszeit von 6 Stunden nur noch der Abbau zuvor starker markierter Proteine autoradiographisch erfaßt wird Danach ware die Abnahme der Silberkorndichte ein Hinweis eher auf Anderungen in der Kinetik als auf eine Abnahme der Proteinsynthese

Da nach kurzer Schallbelastung eine Zunahme der Proteinkonzentration in Innenohrzellen gefunden wird und 85 db Rauschen einen relativ niedrigen Reiz darstellen, weist die Zunahme der Silberkorndichte im Kurz- und Langzeitversuch bei den Einschrankungen der Aussagen von Autoradiogrammen am ehesten auf eine Steigerung der Proteinsynthese hin

Dabei zeigt der Vergleich der maximalen Markierung keine wesentliche Reaktion bei Ganglien- und Bottcherzellen (Tab II) Deren Abweichungen im zeitlichen Verlauf der Silberkorndichte gehen wahrscheinlich auf die

Kinetik des Vorläufers unter der Beschallung zurück. Eine Änderung der Geschwindigkeit und der Menge des axonalen Stofftransportes durch Reizung von Nervenzellen ist bisher nicht beschrieben (Übersicht Lubinska, 1975). Alle übrigen Innenohrgewebe reagieren mit einer Zunahme der Proteinsynthese. Auffallend ist die stärkste Reaktion bei Sulcusepithelien und Fibrozyten des Ligamentum spirale, die schon bei der Untersuchung zur Proteinsynthese unter Streptomycin festgestellt wurde (Richrath und Kraus, 1972). Möglicherweise sprechen diese Zellen auf Innenohrreize besonders stark an, da nach ihrer ribosomalen Syntheserate (Tab. II) zu urteilen unter Belastung eine wesentliche Steigerung der Proteinsynthese möglich ist. Hierfür spricht im ausgewerteten Bereich des Ligamentum spirale ebenfalls der gegenüber angrenzenden Zellen höhere Gehalt der Wurzelzellen an Glutamat-Dehydrogenase (Giebel und Plester, 1972). Die höchste Reaktion ist bei den Haarzellen als unmittelbar gereizter Bestandteil des Innenohres zu erwarten. Im Kurz- und Langzeitversuch ist jedoch nur eine im unteren Durchschnittsbereich liegende Zunahme festzustellen. Damit ist allerdings weder eine zellspezifische Empfindlichkeit bewiesen noch ausgeschlossen.

In der Kinetik ist für das Cytoplasma von Nervenzellen eine „Normalisierung" nach 24 Stunden noch nicht erreicht und bei Zellen der Scala vestibuli sowie bei äußeren Haarzellen nach 8 Stunden noch eine Reaktion festzustellen, während bei den übrigen Innenohrgeweben die Folge der Belastung mit Schall nach 4 Stunden abgeklungen ist.

Beim Markierungsverhalten des Karyoplasmas von Ganglienzellen ergeben sich gegenüber dem des Cytoplasmas keine wesentlichen Abweichungen, während bei Haarzellkernen die Unterschiede zwischen Kontrolle und Beschallung reduziert und zum Teil aufgehoben sind. Die Ursache hierfür liegt wahrscheinlich in der Zusammenfassung einer für die vier Windungen stark schwankenden Anzahl von Ergebnissen.

4.8. WINDUNGSUNTERSCHIEDE BEI HAARZELLEN

Für Nervenzellen des Ganglion spirale cochleae sind morphologische, cytochemische und elektrophysiologische Windungsunterschiede beschrieben. Die Zellgröße nimmt nach apikal ab und basale Ganglienzellen besitzen einen höheren Proteingehalt als die der übrigen Windungen (Hammer, 1956, 1958). Aktionspotentiale lassen sich von apikalen Nervenzellen nur nach tiefen Tonfrequenzen, von basalen nach jeder Frequenz ableiten (Katsuki und Mitarb. 1958). In dieser stärkeren, funktionellen Belastung der I. Windung wird die Ursache für die Zunahme des Eiweißstoffwechsels in den Zellen des Ganglion spirale cochleae von apikal nach basal um 30% vermutet (Koburg und Meyer zum Gottesberge, 1964; Meyer zum Gottesberge und Mitarb. 1965). Demgegenüber findet sich nach 48-stündiger Belastung mit 90 db Rauschen die höchste Aminosäureinkorporation in der 4. Windung (Koburg und Hempel, 1965). Dieser Widerspruch ist nur zu erklären, wenn eine windungsunterschiedliche Kinetik für Aminosäuren angenommen werden konnte oder wenn nach der langdauernden Reizung die Ganglienzellen in den Windungen teils mit Steigerung, teils schon mit einer Depression der Proteinsynthese reagieren. Zumindest für Phonorezeptoren ist eine Erschöpfung der Fermentaktivität nach zweitägiger Beschallung mit einem Dauerton von 85 db beschrieben (Vosteen, 1958).

Gegenüber älteren Untersuchungen erlaubt hier die morphologische Erhaltung der Cochlea nach Kunststoffeinbettung die Auswertung innerer und äußerer Haarzellbereiche aller Windungen. Die Höhe des Cortischen Organs nimmt von der Basilarmembran gemessen für die einzelnen Windungen nach apikal zu. Von basal nach apikal wird für die Phonorezeptoren entsprechend dem Ausmaß bei Ganglienzellen eine Abnahme des Proteinstoffwechsels um ca. 30% gefunden (Tab. I). Für äußere Haarzellen werden die Ergebnisse durch ein identisches Verhalten der cytoplasmatischen

eine Vorverlagerung der Markierungsmaxima und -minima gegenüber Kontrollen festzustellen Bei den Haarzellen und deren Zellkernen sind diese Unterschiede nicht so deutlich

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Dabei zeigt der Vergleich der maximalen Markierung keine wesentliche Reaktion bei Ganglien- und Bottcherzellen (Tab II) Deren Abweichungen im zeitlichen Verlauf der Silberkorndichte gehen wahrscheinlich auf die

Stria vascularis vergleichbare Respirationsrate hinweist (Chou, 1963, dagegen Rauch 1973).

Gegenüber bisherigen Ergebnissen nach Entkalkung und Paraffineinbettung der Felsenbeine (Meyer zum Gottesberge 1961 Koburg und Plester, 1962*a*) zeigen vorliegende Messungen einen deutlich niedrigeren Eiweißumsatz bei fast allen Innenohrzellen Da in dichteren Gewebsbezirken die unspezifische Bindung markierter, freier Aminosäuren stärker ausgeprägt ist als in locker liegenden Zellverbänden und die Relationen für die Stria vascularis und das Limbusepithel mit früheren Befunden weitgehend übereinstimmen konnte durch diesen Artefakt bei Ganglienzellen eine höhere Proteinsynthese vorgetäuscht sein so daß sich für die anderen Gewebe zu niedrige Relationen ergeben Dagegen spricht jedoch der bei beiden Methoden fast identische Eiweißumsatz für die nur aus zwei Zellagen bestehende Reißnersche Membran für die präparatorisch die gleichen Bedingungen anzunehmen sind wie für die Zellen des Cortischen Organs Die Unterschiede jetziger Ergebnisse gegenüber früheren dürften bei besserer morphologischer Erhaltung durch die geringere Schrumpfung der Gewebe während der Aufbereitung zu erklären sein

Der Vergleich mit Meßergebnissen des RNA-Gehaltes zeigt daß die cytoplasmatische RNA-Konzentration (Kraus 1970) mit dem Eiweißumsatz der Innenohrzellen (Tab II Spalte 1, 6 und 7) für einzelne Gewebe nicht übereinstimmt Böttcher-, Hensenzellen und Reißnersche Membran zeigen im Vergleich zu Ganglienzellen einen bis vierfach überhöhten Eiweißumsatz bezogen auf den cytoplasmatischen RNA Gehalt während für Sulcusepithelien Fibrozyten des Ligamentum spirale und Zellen der tympanalen Belegschicht der Eiweißumsatz bis zur Hälfte reduziert ist Bei Sinnes und Deiterschen Zellen korrelieren jedoch Silberkorndichte und RNA Gehalt Da mit der Gallocyanin-Chromalaun-Färbung im Cytoplasma überwiegend die ribosomale RNA erfaßt wird ist der Quotient aus Aminosäureinkorporation und RNA-Konzentration ein relatives Maß für die ribosomale Aktivität bei der Proteinsynthese (vgl Kraus, 1970) Beim Vergleich dieser Syntheseraten basierend auf früheren und jetzigen Ergebnissen zum Eiweißumsatz zeigt sich daß der Durchschnitt aus allen Geweben von 2 6 auf 1 26 nach Eponeinbettung zurückgegangen ist und sich somit der Eiweißumsatz generell dem RNA-Gehalt der Innenohrzellen nähert Bis auf Böttcherzellen sowie Hensenzellen überschreiten die Abweichungen ±50% nicht Insbesondere die früher bei Haarzellen gefundene Überhöhung der ribosomalen Syntheserate im Vergleich zu Ganglienzellen und Striaepithelien läßt sich nicht mehr feststellen Zu berücksichtigen ist jedoch daß Meßergebnisse von entkalkten Schnecken mit denen von unentkalkten verglichen werden Neben Unterschieden in der morphologischen Erhaltung gehen RNA Verluste durch Säurebehandlung in die Messungen mit ein (Übersicht Schultze, 1969)

Auf toxische und adäquate Belastungen scheinen Haarzellen besonders stark mit einer Funktionseinschränkung zu reagieren, wofür eine erhöhte ribosomale Syntheserate einen prädisponierenden Faktor darstellen könnte (Kraus 1970) Demgegenüber können vorliegende autoradiographische Ergebnisse und frühere zur Wirkung von Streptomycin auf die Proteinsynthese beim Meerschweinchen (Richrath und Kraus 1972) eine spezifische Empfindlichkeit der Sinneszellen nicht bestätigen Auch hieraus kann gefolgert werden, daß wesentliche Unterschiede zwischen der ribosomalen Syntheserate von Haarzellen und der von Nervenzellen des Ganglion spirale nicht bestehen

Da die Sinneszellen in ihrer metabolischen Versorgung weitgehend auf Diffusion angewiesen sind ist letztlich für ihre Funktion die Stoffwechsellage sekretorischer oder resorptiver Gewebebereiche in der Cochlea entscheidend Auffallend ist die Auswirkung von Reizen auf das äußere Sulcusepithel, dessen Versorgung wie bei allen Epithelien von der Basis aus anzunehmen ist Dieser entspricht

jedoch im ausgewerteten Bereich des Ligamentum spirale zum Teil die tiefreichende Schicht der Wurzelzellen Insgesamt zeigt sich auch hier nach Reizen autoradiographisch eine starkere Reaktion, so daß Auswirkungen auf die Zusammensetzung der Perilymphe denkbar sind, da die Interzellularspalten des Ligamentum spirale mit den Flussigkeitsraumen beider Scalen in direkter Verbindung stehen (v Illberg, 1968) Ob der Stoffwechsel der Sinneszellen hierdurch ebenfalls betroffen ist, laßt sich nicht entscheiden, die Angaben über eine freie Passage zwischen Perilymphe und Cortilymphe sind widerspruchlich (Angelborg, 1974)

Erhaltung und Funktion einer Zelle sind von der Proteinsynthese abhangig, da selbst in Nervenzellen bisher keine stabilen Proteine festgestellt werden konnten Ebenso wird mit Hilfe von Proteinen der zellspezifisch verfugbare Umfang der genetischen Informationen festgelegt und deren Umsetzung in Eiweiße ist nur in der Aggregation von Ribonukleinsauren mit spezifischen Proteinanteilen moglich Die Abnahme des Proteingehaltes durch starkere Reize konnte demnach einen Faktor in der Storung des Zellstoffwechsels darstellen, bevor am Cortischen Organ morphologisch faßbare Schaden auftreten Eine Schwellenabwanderung soll beim Meerschweinchen erst bei starkeren Schallpegeln als bei 85 db auftreten (Schnieder, 1974), so daß die Steigerung der Proteinsynthese wahrscheinlich nur ein *Vorstadium der temporaren Schwellenabwanderung* darstellt Die Kinetik der Eiweißsynthese in der Cochlea wie die Abnahme des Protein- und Fermentgehaltes (Vosteen, 1958) stimmen insoweit mit der Entwicklung des Schalltraumas uberein die Erschopfung des Proteingehaltes entspricht eher dem permanenten Schwellenverfall nach Schallexposition als der temporaren Schwellenabwanderung

Aus vorliegenden Ergebnissen ist zu schließen, daß der Apparat der Proteinsynthese der Innenohrzellen, wahrscheinlich aber auch die Membranpermeabilitat und der Aminosaurepool, schon auf relativ geringe Schallreize deutlich reagiert

5. Anhang zur Methodik (Reflexionsphotometrie)

5.1. EINLEITUNG

Bisher wurde die Reflexionsphotometrie nur zur Messung von Silberkörnern bei Einzelzellen benutzt (Dormer und Mitarb., 1966, Dormer, 1967).

An ungefärbten Autoradiogrammen kunststoffeingebetteter Schnecken wurde geprüft, ob durch Änderung des Auflichtstrahlenganges und der Präparationsmethode auch über histologischen Schnitten eine lineare Beziehung zwischen Anzahl und reflektierter Lichtintensität der Silberkörner erreicht werden kann und die photometrische Auswertung dieser Autoradiogramme mit Hilfe einer Regressionsfunktion möglich ist.

5.2. GERÄTE UND METHODEN

Die von Autoradiogrammen reflektierte Lichtintensität wurde mit einem handelsüblichen *Auflicht-Mikroskopphotometer* gemessen. Benutzt wurde ein Phasenkontrast-Fluoritobjektiv 100× (n.A. 1,3, Ölimmersion). Über einen Planglasreflektor erfolgte die Hellfeldauflichtbeleuchtung, deren Apertur durch eine fixe Blende im Auflichtstrahlengang auf ca. 0,3 reduziert war. In der Objektebene betrugen Leuchtfeld- und Meßblendendurchmesser 5,7 µm bzw. 4,8 µm. Die Meßstellen wurden im Durchlicht-Phasenkontrast aufgesucht, dieser Strahlengang konnte unter dem Kondensor unterbrochen werden. Auflicht- und Durchlichtstrahlengang waren nach dem Köhlerschen Prinzip justiert und enthielten beide auf der Beleuchtungsseite ein Interferenzfilter (λ_{max} 539 nm, T_{max} 61%, HW 94,5 nm). Der photoelektrische Teil bestand aus einem Sekundärelektronenvervielfacher (Typ RCA 1 P 28) mit Meßverstärker und digitaler Ziffernanzeigeeinheit. Als Standard zur Eichung der Meßempfindlichkeit diente ein Glasprisma (Leitz R 9, $n_{546 nm}$ = 1,8978), dessen Reflexionsvermögen in Öl nahe dem der untersuchten Autoradiogramme lag.

5.3. ERG

Im Durchlicht-Phasenl
turen der in Epon ein
Cochlea mühelos zu d
heben sich durch eine
Cytoplasma ab, bei de
die gut sichtbare Ker
Körner liegen fast übe
einer Ebene der Film
auch bei hoher Marki
den Ganglienzellen, in
ser im Auflicht, ein
Trotz Hellfeldbeleucht
lichtstrahlengang ein
Silberkörner erheblich
der Untergrund. In de
berkornschicht läßt sic
gut abbilden. Nach Fe
tes" mit Hilfe des Sta
flexion der Optik des
0,16%. Die Präparate
helligkeit von ca. 5%,
kierten Schnitten oder
gemessen. Die Frontl
densors zeigt keinen
Bei einer numerisch
beleuchtung von 0,
parallel einfallender
Fehler durch Streul
überwiegend von S

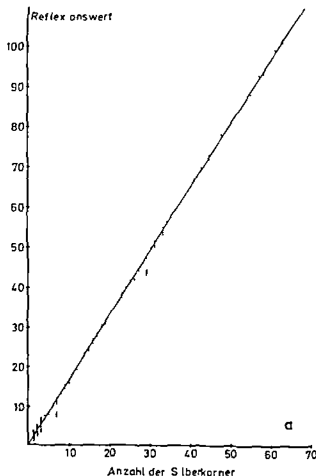


Abb 8a Beziehung zwischen reflektierter Lichtintensitat und Anzahl der Silberkorner uber Zellen im Gewebsverband Regressionsgerade eingezeichnet Untergrundhelligkeit der Leerstellen der Praparate abgezogen

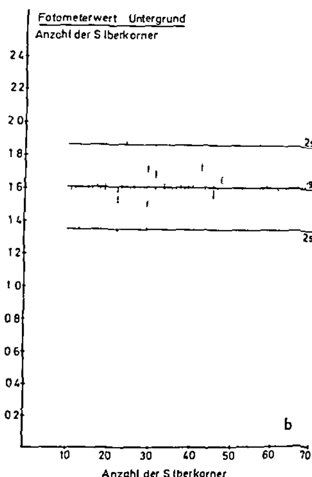


Abb 8b Mittelwert und 2 s Streuung der Reflexionsintensitat des einzelnen Silberkorns Abszisse gezahlte Silberkorndichte pro Meßfeld Ordinate Quotient aus Photometerwert (nach Abzug der Untergrundhelligkeit) und Anzahl der Silberkorner pro Meßfeld (nach Dormer und Mitarb 1966)

anteils zu erwarten um den in der Objektebene die Leuchtfeldblende größer als die Meßblende ist Wird jedoch bei der angegebenen Blendenkombination ein isoliert liegendes *Silberkorn schrittweise durch das Meßfeld* gefuhrt so sinkt der angezeigte Reflexionswert auf den des Hintergrundes sobald das Silberkorn die Grenzen des Meßfeldes nach außen uberschritten hat Die Beziehung zwischen Reflexionswert und im Meßfeld gezahlter Silberkorner ergibt sich aus Abb 8*a* Der Reflexionswert des silberkornfreien Untergrundes ist von den ca 500 Meßwerten unterschiedlich dicht markierter Gewebsareale abgezogen Wird jeder Meßwert durch die gezahlte Silberkornzahl dividiert und der Quotient in Anhangigkeit von der Silberkornzahl eingetragen (Abb 8*b*) so laßt sich die Große des gesamten Meßfehlers abschatzen (Dormer *und Mitarb 1966) Hierbei zeigen sich große* Schwankungen im Bereich geringer Silberkorndichten wahrend ab einer Anzahl von 10 Silberkornern pro Meßfeld eine lineare Beziehung festzustellen ist Der ab hier aus der 2 s Grenze berechnete statistische Fehler betragt ca 16% Wird der visuelle Zahlfehler mit 10% angesetzt verbleibt für die Meßeinrichtung bei vorliegenden Praparaten ein Restfehler von ca 6%

Die statistische Sicherung wurde nach den

üblichen Kriterien für Regressionsfunktionen durchgeführt (Sachs, 1972)

5.4 DISKUSSION

Die von Dormer und Mitarb (1966) angegebene, photometrische Methode ist auf die Auswertung von Einzelzell-Autoradiogrammen begrenzt, da Streulicht markierter, eng gruppierter Zellen in unkontrollierbarem Ausmaß in die Messungen eingeht und dieser Fehler die Auswertung von Autoradiogrammen histologischer Schnitte weitgehend ausschließt Zur Differenzierung von Einzelzellen ist eine nachträgliche Färbung notwendig Bei seinen Autoradiogrammen ergibt sich der energetisch günstigste Abstand zwischen Untergrundhelligkeit und Reflexionsvermögen der Silberkörner bei identischer Beleuchtungs- und Beobachtungsapertur

Die in der Histologie üblichen Farbstoffe sind bei Auflichtbeleuchtung ausgesprochene Reflektoren (Westphal, 1963) Dagegen wird bei den hier untersuchten Präparaten allein durch Verzicht auf eine Färbung ein ausreichender Abstand der Meßwerte von der Untergrundhelligkeit erreicht und der Fehler durch Reflexion an inhomogen verteilten Farbresten in der Photoemulsion und im Schnitt vermieden

Eine möglichst genaue Abbildung der Silberkörner im Meßfeld sowie umgekehrt der Leuchtfeldblende in der Meßebene erfordert übereinstimmende Brechungsindices aller zwischenliegenden Medien Dadurch werden kontrastmindernde Reflexionen an Grenzflächen reduziert Annähernd lassen sich diese Bedingungen nur durch Photoemulsionen verwirklichen deren Schichtdicke gering ist und deren Gelatine (n_{D20} = 1 516–1,534) im Brechungsindex nicht zu sehr von dem der anderen Medien abweicht Geringe Abweichungen machen sich jedoch bei Vergleichpräparaten mit dem Strippingfilm Kodak AR 10 wegen der Dicke von 10 μm durch Aufhellung des Meßfeldes deutlicher bemerkbar als nach dünner Beschichtung mit der Emulsion Nuc 715

Durch Schrumpfung und Quellen histologischer Schnitte nach Paraffineinbettung liegen die Silberkörner von Autoradiogrammen in unterschiedlichen Ebenen, deren Abstand den Tiefenschärfenbereich von Ölimmersionen der n A 1 3 übersteigt Durch Partikel außerhalb des Focus wird die Verteilung der Lichtintensität beeinflußt Hierauf beruhende Fehler lassen sich in der Durchlichtphotometrie durch absuchende Punktmessungen über der Gesamtfläche des Objektes eliminieren (Sandritter, 1958), dagegen gehen sie bei Reflexionsmessungen mit großen Photometerblenden in die Messungen ein Wegen der ebenen Oberfläche von Kunststoffschnitten macht sich dieser Fehler bei den durchgeführten Untersuchungen weniger bemerkbar

Immersionen der Maßstabzahl 100 besitzen numerische Aperturen von 1,2 bis 1,4 Bei offener Aperturblende der Auflichtbeleuchtung beträgt der Incidenzwinkel ca 60°, bei Reflexionsmessungen stellt sich ein systematischer Meßfehler durch Streulicht ein In der Durchlichtphotometrie können Streulichtwerte durch niedrige Kondensoraperturen gemindert werden (Sandritter, 1958) Ähnliche Bedingungen gelten auch für die Auflichtphotometrie und wahrscheinlich ist die Ausnutzung der vollen Beleuchtungsapertur für das Streulicht benachbarter Silberkorngruppen verantwortlich Wird der Einfallwinkel der Auflichtbeleuchtung von ca 60° (Dormer und Mitarb, 1966) auf ca 12° (ca 0,3 n A) verringert und dadurch das Lichtbündel weitgehend parallel, so läßt sich der Streulichtanteil eines einzelnen Silberkornes außerhalb der Photometerblende nicht mehr messen Danach ist die photometrische Auswertung aneinandergrenzender Meßfelder nur bei niedriger Kondensorapertur möglich

Das Reflexionsvermögen an Grenzflächen optisch unterschiedlicher Medien richtet sich nach der Beerschen Formel und erreicht bei Licht der Wellenlänge 539 nm für Silber fast 100% (Pohl, 1954) gegenüber ca. 4% für das

umgebende Medium bei einem Brechungsindex von $n_{D^{20°}} \approx 1{,}53$ Hieraus erklart sich der Dunkelfeldeffekt bei reflexionsmikroskopischer Betrachtung von Autoradiogrammen

Das Spektrum der Kristallgroßenverteilung von Kernspuremulsion ist relativ eng, der mittlere Durchmesser betragt bei der Photoemulsion Nuc 715 1 500 Å Die Große der Kristalle und daraus entwickelter Silberkorner liegt an der Grenze des lichtmikroskopischen Auflosungsvermogens Obwohl sich gegenuber der Durchlichtbeleuchtung die Strukturwiedergabe mikroskopischer Bilder von Autoradiogrammen durch Auflichtbeleuchtung erheblich steigern laßt, werden die einzelnen Silberkorner lichtmikroskopisch nicht mehr objektahnlich abgebildet Diese Voraussetzung fur durchlichtphotometrische Absorptionsmessungen an Partikeln besitzt in der Reflexionsphotometrie von Autoradiogrammen wahrscheinlich nicht diese Bedeutung, vom einzelnen Partikel wirkt sich überwiegend r die von der Oberflachengroße abhangige flexionsintensitat aus Zum Fehler durch terschiedliche Oberflachengroßen mussen i Reflexionsmessungen von Autoradioimmen Abweichungen durch die ungeordte Orientierung der reflektierenden Flachen diert werden Mit Objektiven hochster nu:rischer Apertur konnen jedoch nur Lichtahlen bis zu einem maximalen Reflexionsnkel von ca 60° erfaßt werden Der dadurch dingte Fehler ist nicht genau abzuschatzen, ua fur vergleichende Messungen einzelner Silberkorner die Großenunterschiede nicht bestimmt werden konnen Beide Faktoren sind vermutlich fur die starke Streuung der Meßwerte bei geringer Markierungsdichte verantwortlich (Abb 8*b*) und legen die untere Grenze bei der photometrischen Auswertung von Autoradiogrammen mit der Photoemulsion Nuc 715 auf ca 6 Silberkorner pro 10 μm^2 fest Oberhalb dieser Markierungsdichte betragt der durch Reflexionsrichtung und Silberkorngroße bedingte Fehler ca 6%

Von einer bestimmten Silberkorndichte ab ist damit zu rechnen, daß die Proportionalitat zwischen Tritiumzerfall und Anzahl der entwickelten Silberkorner verschoben wird, da ein einzelner Silberbromidkristall mehrfach von β Teilchen getroffen werden kann Gegenüber einem durchschnittlichen Kristalldurchmesser von 0,3 μm beim AR 10 Strippingfilm ist bei der Emulsion Nuc 715 mit einem mittleren Korndurchmesser von 0,15 μm eine Herabsetzung dieses Koinzidenzfehlers (Dormer, 1967) zu erwarten, zumal bei den vorliegenden Autoradiogrammen mit maximal 70 Silberkornern pro Meßfeld nur ca 7,5% der bei Sattigung moglichen Anzahl von Silberkornern einer geschlossenen, einschichtigen Lage (ca 950 Silberkorner/18 μm^2) erreicht sind Eine Korrektur unterblieb aus diesem Grunde

Die Einstellung der Meßempfindlichkeit mit Hilfe eines Glasprismas als Standard ist jederzeit reproduzierbar und schließt Fehler objektahnlicher Standards, wie Inhomogenitat der Silberkornverteilung und ungenaue Reproduzierbarkeit der Eichstelle (Dormer und Mitarb 1966) weitgehend aus

6. Zusammenfassung

Autoradiographisch wurde nach intraperitonealer Injektion die Inkorporation von ³H-DL-Leucin in die Cochlea junger Meerschweinchen untersucht. Lichtmikroskopisch ergab sich nach Eponeinbettung ohne Schneckenentkalkung eine reproduzierbare morphologische Erhaltung, die die Differenzierung der Zellen des Cortischen Organs in allen Windungen erlaubte Elektronenmikroskopisch zeigten nur das Spiralganglion und die Stria vascularis eine ausreichende autoradiographische Markierungsdichte Bei Nervenzellen waren das endoplasmatische Reticulum und Vakuolen des Golgi-Komplexes markiert Die mit längerer Versuchszeit zunehmende Silberkorndichte über Mitochondrien, Ursprungskegeln und Axonen dürfte sich durch die intrazellulare Wanderung von Proteinen erklären Die starke autoradiographische Reaktion über den Nucleoli und der Perichromatinregion läßt einen hohen Umsatz an Ribonucleoproteinen vermuten Der bisher im Lichtmikroskop autoradiographisch erfaßte Eiweißstoffwechsel tieferer Schichten der Stria vascularis entspricht wegen der stärkeren Markierung eher dem chromophiler Zellen In äußeren Haarzellen war das Ergastoplasma nahe der Zellmembran in der Eiweißsynthese aktiver als der Hensenkörper Fibrillare Strukturen in Stützzellen des Cortischen Organs sowie in der Basilarmembran junger Meerschweinchen zeigten eine Aminosäureinkorporation, die der numerischen Zunahme von Tonofibrillen bis zur Adoleszenz entspricht

In einem Kurzversuch wurde das rechte Ohr von 12 narkotisierten Meerschweinchen nach Injektion von ³H-DL-Leucin mit 85 db Rauschen beschallt und bis zu 4 Stunden nach Tracerapplikation die Silberkorndichte der Gewebe beider Basalwindungen bestimmt. Die Markierungsdichte im Cytoplasma der Sinneszellen erreichte lediglich 1/4 der von Ganglienzellen Im zeitlichen Verlauf wurde nach 2 Stunden ein Maximum erreicht; diese Silberkorndichte blieb über 1 Stunde erhalten und fiel nach 4 Stunden auf die Ausgangswerte zurück Die beschallte rechte Seite zeigte zu allen Versuchszeiten eine stärkere Aminosäureaufnahme, statistisch signifikante Unterschiede fanden sich jedoch nur bei geringer markierten Geweben. Die Ursache der geringen Seitenunterschiede dürfte in der gleichzeitigen Mitreizung des linken Ohres über die Knochenleitung liegen.

In einem Langzeitversuch wurden 24 hungernde Meerschweinchen nach Injektion von ³H-DL-Leucin mit 35 dB weißem Rauschen beschallt und die Silberkorndichte über den Geweben der Basalwindung in den Intervallen 1, 2, 3, 4, 8 und 24 Stunden nach Tracerapplikation bestimmt. 12 Stunden lang vor der Injektion wurde die eine Hälfte der Tiergruppe 35 dB (=Kontrolle) Rauschen, die andere Hälfte 85 db (=Beschallung) ausgesetzt Bei Kontrollen (35 db) zeigten die Kurven der Silberkorndichte angedeutet einen biphasischen Verlauf mit einem Inkorporationsmaximum nach 2–3 Stunden, einem Abfallen nach 8 Stunden und einem Wiederanstieg zum 24-Stunden-Wert.

In beiden Versuchen zeigte sich somit ein relativ später und breiter Markierungsgipfel Damit weicht die Kinetik der Proteinsynthese des Innenohres von der innerer Organe ab und entspricht eher der des Gehirns Die Ursache

des Wiederanstiegs im Langzeitversuch ist in der Reutilisation von Aminosauren aus dem Proteinabbau peripherer Organe anzunehmen, da wegen des zunehmenden Hungerzustandes der Tiere wahrend des Versuches die metabolische Versorgung lebenswichtiger Systeme zu Lasten von Leber und Muskulatur geht

Nach Beschallung (85 db, 12 Stunden) zeigten alle Gewebe des Langzeitversuches eine hohere Inkorporation und eine zeitliche Vorverlagerung der Extremwerte Die Abweichungen in der Markierungskinetik hielten bei Ganglienzellen bis 24 Stunden an, wahrend bei den ubrigen Innenohrzellen Unterschiede zwischen Kontrolle und Beschallung nach 4 und 8 Stunden nicht mehr festzustellen waren Die prozentual starkste Reaktion wurde bei den Fibrozyten des Ligamentum spirale und bei Sulcusepithelien festgestellt, die Reaktion bei Sinneszellen lag unter dem Durchschnitt Als Ursache der erhohten Inkorporation kommen in Frage Zunahme der Durchblutung, der Konzentration von ^{3}H-Leucin in den Lymphen, der Zellpermeabilitat und Abweichungen im Aminosaurepool der Zellen Am wahrscheinlichsten ist jedoch eine echte Steigerung der Proteinsynthese

Die prozentualen Relationen des Eiweißumsatzes stimmen mit dem relativen RNA-Gehalt der einzelnen Innenohrgewebe weitgehend uberein Danach scheidet eine erhohte ribosomale Syntheserate als pradisponierend für die Empfindlichkeit von Haarzellen gegenuber Noxen aus

Beim Vergleich der verschiedenen Windungen nahm die Silberkorndichte uber dem Cytoplasma innerer und außerer Haarzellen von basal nach apikal um 30% ab Eine Vergrößerung der Haarzellkerne war lediglich in der ersten Stunde nach Beschallung festzustellen und durfte ein Hinweis auf die Einhaltung physiologischer Reizintensitaten sein

Die Befunde zeigen, daß in der Cochlea schon nach relativ niedrigen Reizintensitaten mit einer Anderung in der Kinetik der Proteinsynthese, wahrscheinlich aber auch der metabolischen Versorgung, der Membranpermeabilitat und der intrazellularen Reserven gerechnet werden muß

Die reflexionsphotometrische Bestimmung der Silberkorndichte ist bisher nur an Einzelzellautoradiogrammen durchgefuhrt worden Nach Kunststoffeinbettung und Einengung der Beleuchtungsapertur auf 0 3 zeigte sich auch uber Zellverbanden eine lineare Beziehung zwischen Silberkorndichte und reflektierter Lichtintensitat

7. Summary

After intraperitoneal injection the incorporation of ^{3}H-DL-leucine in the cochlea of young guinea pigs was investigated by autoradiography. As demonstrated by light microscopic examination a reproducible morphological preservation could be achieved by embedding the inner ear in Epon without decalcification of the temporal bone, thus permitting discrimination of the cells of the organ of Corti in each of the four turns. The electron microscopic autoradiographs indicated sufficient grain density only over spiral ganglion cells and the stria vascularis. In nerve cells the endoplasmatic reticulum and the vacuoles of the Golgi complex were labeled. Increasing duration of experiments resulted in a gradual rise of grain density over the mitochondria, the axon hillocks and the axons. This can be explained by intracellular migration of proteins. The intense autoradiographical reaction over the nucleoli and the perichromatin regions suggested a high turnover of ribonucleoproteins. In deeper layers of the stria vascularis the protein metabolism previously studied autoradiographically by means of light microscopy corresponds rather to the chromophile cells because of intenser marking. In outer hair cells the ergastoplasm lining the cell wall was more active in protein synthesis than the Hensen body. The fibrillar structures in supporting cells of the organ of Corti and in the basilar membrane of young guinea pigs showed an incorporation of amino acid which corresponds to the numerical increase of fibrils up to adolescence.

In a short time experiment on 12 anaesthetized guinea pigs with the right ears exposed to noise of 85 db up to four hours after the injection of ^{3}H-DL-leucine the grain density over the cells of the basal turn of both sides were measured. Over the cytoplasm of sensory cells the grain density reached only a quarter of that of ganglion cells. A maximum was reached after two hours, this grain density was preserved for one hour and fell down to the initial values after four hours. Compared to the left ear the right one, which had been exposed to noise, showed a greater amino acid incorporation at any time investigated during the experiment. Statistically significant differences could be observed only in less labeled tissues. The reason for only slight differences between the two sides might be the simultaneous stimulation of the left ear by bone conduction.

In a long time experiment a group of 24 starving guinea pigs was exposed to white noise of 35 db SPL after ^{3}H-DL-leucine injection. The grain density in the basal turn of each cochlea was measured 1, 2, 3, 4, 8 and 24 hours after application of the tracer. 12 hours prior to injection one half of the group was exposed to noise of 35 db SPL for control purposes ("Kontrolle"), while the other half was subjected to noise of 85 db SPL ("Beschallung"). The curves of grain density derived from experiments on the control group indicated a biphasic development with a maximum of incorporation after 2–3 hours, a decrease after 8 hours, and a repeated increase in the values after 24 hours.

Thus, in finding a relatively late and broad peak of marking in both experiments the kinetics of the protein synthesis in the inner ear could be demonstrated to be different from that of internal organs and to correspond rather to that of the brain. The reutilisation of

amino acids derived from proteolysis in the peripheral organs is assumed to be the reason for the repeated increase in the long time experiment, because as a result of increasing state of starvation during the experiment the liver or the muscles supply metabolically vital organs

After exposure to noise (85 db, 12 hours) all tissues in the long time experiment showed a higher incorporation of the labeled amino acid and earlier maxima and minima of grain density compared to the control group The differences in the kinetics of grain density persisted up to 24 hours in the nerve cells, whereas in other cells of the inner ear the differences between the control group and that exposed to noise had disappeared after 4 or 8 hours The highest reaction to the acoustic stimulus in terms of percentage was detected in fibrocytes of the ligamentum spirale and in epithelial cells of the sulcus In hair cells the reaction lay below the mean value The increased incorporation might be caused by increased blood supply, concentration of ^{3}H leucine in ymphs, cell permeability or differences e amino acid pool of the cells, but most / is a real increase in the cochlear protein iesis

The relations in percentage of the protein metabolism in the different tissues of the inner ear agreed substantially with the relative values of RNA concentration Accordingly an increased rate of ribosomal peptid synthesis can be excluded as a predisposing factor for the sensitivity of hair cells to noxae

Comparing the different cochlear turns the grain density over the cytoplasm of inner and outer hair cells decreased from the basal to the apical turn by about 30% An enlargement of the hair cell nuclei could be detected only one hour after noise exposure This might indicate that the intensity of the stimulus was kept within physiological range

The findings show that in the cochlea even relatively low acoustic intensities may alter the kinetics of the protein synthesis, probably also the metabolical supply, the membrane permeability and the intracellular reserves

The measurement of silver grain density with an incident light microscopic photometer in bright field has previously been carried out on single cell autoradiographs only After embedding in plastic and confining condensor aperture to 0 3 a linear relationship between grain density and reflected light intensity could be detected over layers of tissue, too

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SUPPLEMENT 353

)antification of the Threshold ıd Latency Parameters of the \coustic Reflex in Humans

BY

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Table I *Effect of the type of acoustic stimuli on the threshold of the acoustic reflex*

	White noise	Pure tones 500 Hz	1 000 Hz	2 000 Hz	4 000 Hz	Tonal mixtures
Dallos (1964)	79 5 dB SPL					
Habener & Snyder (1974)		83 3 dB SPL	83 7 dB SPL	84 9 dB SPL	83 1 dB SPL	
Jerger et al (1972)		[illegible]	[illegible]	[illegible]	[illegible]	
Niemeyer & Sesterhenn (1974)	68 5 dB SPL					67 2 dB SPL
Peterson & Lidén (1972)	77 2 dB SPL					

Table II *Effect of stimulus duration and age of subject on the thresholds of the acoustic reflex*

	Stimulus	Threshold difference
Djupesland & Zwislocki (1971)	2 000 Hz	10 msec–1 000 msec acoustic stimuli 35 dB difference in ART
Woodford et al (1975)	500 Hz 1 000 Hz 2 000 Hz 3 000 Hz 4 000 Hz	10 msec–500 msec acoustic stimuli 30 dB difference in ART

Age of subject (Habener & Snyder 1974)

	Frequency (Hz)	Age group years 0–9	10–19	20–29	30–39	40–49	50–59	60–69
Threshold dB SL	500	95 0	89 2	80 3	85 7	82 1	87 8	79 1
	1 000	94 5	85 7	80 7	83 8	81 8	84 3	77 5
	2 000	96 5	88 5	83 0	87 3	82 1	83 6	73 3
	4 000	101 0	88 1	78 5	84 6	83 7	77 8	69 0

gested by Niemeyer and Sesterhenn (1974) and [illegible] et al (1974), may have important [illegible] application in testing infants and [illegible] difficult to test patients Table I summarizes acoustic reflex threshold data obtained by several researchers

Quantitative threshold measurement may be accomplished by connecting a strip chart recorder or oscilloscope to an electroacoustic impedance bridge The difficulty encountered in this procedure is definition of the point of change in the baseline tracing which constitutes threshold Møller (1974) stated that a specific response 10% of maximal obtainable impedance change should be judged as threshold of the acoustic reflex because this measure has better reproducibility than absolute threshold determination Use of graphic recording technique provides a method for quantitative analysis of specific temporal characteristics of the acoustic reflex (Hall and Jerger, 1976)

Amplitude of the acoustic reflex

The amplitude of the acoustic reflex has a dynamic range of 30 dB between threshold level and saturation threshold [1] It is both frequency and age dependent Amplitude of the reflex is greatest at 2 000 Hz and least at 4 000 Hz The population in the age span of 20–40 years exhibits the most consistent amplitude at frequencies of 500, 1 000, 2 000 and 4 000 Hz (Jerger, 1972, Habener and Snyder, 1974)

The extent of impedance change at the tympanic membrane when measured by an acoustic impedance bridge is a direct reflection of the magnitude of the acoustic reflex Bekesy (1960) has shown the resonance peak of the external auditory meatus to be 2 500 Hz with a sharp decrease in resonance at adjacent

[1] Dynamic range is defined as the range in dB where the strength of the response (amplitude size of the acoustic reflex) increases with the magnitude of the stimulus in a linear fashion (Dallos 1964)

frequencies This is important since the resonance characteristics of the ear canal enhance the input pressure at the ear drum resulting in an apparent stronger muscle contraction, and thus a greater impedance change The contraction of the middle ear muscles changes the mechanical configuration of the external auditory canal and the middle ear, shifting the resonant frequency Consequently, not only does an impedance change occur due to changes in mass and stiffness of the mechanical system but also to changes in its resonant frequency (Mundie, 1963, McPherson and Miller, 1975)

Acoustic reflex decay

Decay of the acoustic reflex is the time, in seconds that is required for the response amplitude to be reduced by 50% The precise mechanism of decay is not known but the frequency dependent characteristics of the acoustic reflex decay indicates the mechanism is in the afferent protion of the acoustic reflex arc (Anderson et al 1970) Such decay has been shown to exist in normal ears centered around 4 000 Hz However it is virtually non existent at 500 and 1 000 Hz (Habener and Synder, 1974)

Several researchers have shown that the 4 000 Hz area of the basilar membrane is the area most sensitive to damage by noise, drugs and other ototoxic agents (Johnsson, 1971, Johnsson and Hawkins 1972*a* 1972*b*) This may be a partial explanation for abnormalities of the acoustic reflex at 4 000 Hz in normal hearing individuals The reduction of neural elements seen in such cases in absence of any definable pathological state may explain reflex decay in normal hearing individuals at 4 000 Hz

Rise time of the acoustic reflex

A review of the bioacoustic literature regarding the rise time of the acoustic reflex indicates that this parameter has been studied as part of the initial response of the acoustic reflex. Rise time is defined as the time for a biological response to change from 10% to 90% of maximum response amplitude This same definition will be used to describe the rise time of the acoustic reflex

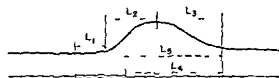
In several animal experiments, Borg (1972, 1972*a*) found that rise time of the response was faster than decay time and that the time course and rise time of the reflex was dependent on the level of stimulation

Colletti (1974, 1975) used a strip chart recorder connected to the output of an impedance bridge to study the acoustic reflex in a normal and abnormal human population Colletti observed a slow rise in some patients with multiple sclerosis This alteration in rise time differs markedly from normal and cochlear impaired ears which exhibited a rapid rise time once muscle contraction began This change in acoustic reflex rise time should be studied further as it relates to acoustic reflex latency As such, it is most important for it to be defined accurately in a quantitative manner

Acoustic reflex latency

Latency is the time, in seconds, it takes a biological system to respond to an appropriate stimuli Latency of the acoustic reflex is the time taken for the middle ear muscles to contract following acoustic stimulation Some researchers (Moller, 1972, 1974, Borg, 1972, Lidén et al , 1974) describe latency as the time, in seconds, from stimulus onset to the time when the acoustic reflex has attained 10% of maximum amplitude Colletti (1974, 1975) defines latency as the period from signal onset to 5% of maximum impedance change, while Sunderland (1974) and Strasser (1975) have suggested measuring latency from signal onset to the beginning of impedance change Consequently, it becomes awkward to compare results from various reports

Latency of the acoustic reflex has b[illegible] measured by direct observation (Lus[illegible] 1929), electromyography (Djupesland, [illegible] Jepsen, 1963), recording of the [illegible] microphonic, and observation of ac[illegible]



L_1 latency from onset of stimulus to initial reflex response

L_2 latency from initial response to peak of the response

L_3 latency from response peak to point where reflex reaches 95% return to baseline

L_4 latency from cessation of stimulus to 95% return to baseline

L_5 total response time L_2 plus L_3

Fig 2 Definitions of latency parameters of the acoustic reflex From Norris (1974)

pedance change (Dallos, 1964) Using acoustic impedance change to measure acoustic reflex latency in human subjects, Dallos (1964, 1973) found the duration of the latent period was inversely proportional to the strength of the acoustic stimulus Additionally, he reported a variation in individual response latencies These qualitative observations are consistent with data reported by Borg (1972, 1972*a*) in animal experiments Dallos described the acoustic reflex as an asymetrical response, is it is a nonlinear response whose teristics depend on the direction (in ise or decrease) and magnitude of the eliciting stimulus The 'on' response of the acoustic reflex is nonlinear while the 'off' response behaves in a linear fashion (Dallos, 1964) The fact that the system is nonlinear means the contraction process differs from the relaxation process and can be studied and discussed separately Recent research has indicated that latency parameters contain important biologic information and can be used as sensitive indicators of disease states (Colletti 1974, 1975 Norris 1974 Strasser 1975)

Norris et al (1974) used an ordered series of latency measures in an effort to find a method of evaluating differences between a cochlear impaired and a normal population Their approach is graphically illustrated in Fig 2 and includes definition of five different latency values Latencies were defined as follows L_1 latency from onset of stimulus to initial reflex response, L_2, latency from initial response to peak of the response, L_3 latency from response peak to point where reflex reaches 95% return to baseline, L_4, latency from cessation of stimulus to 95% return to baseline, L_5, total response time, L_2 plus L_3 Norris et al (1974) elicited the acoustic reflex with a 1000 Hz stimulus at an intensity 10 dB above clinical threshold Recordings were made using a Madsen electroacoustic impedance bridge and a two channel strip recorder Latencies were measured from stimulus onset The chief difference between the two populations studied was in relaxation time of the reflex The "cochlear impaired" population showed a significant difference in L_3, L_4 and L_5 values, exhibiting longer latencies in all cases Norris felt L_3 (latency from response peak to point where reflex reaches 95% return to baseline) was the key variable since all contributions of the contraction phase had been eliminated Norris hypothesized that the cochlear impaired ear showed delay in neural response to cessation of the stimulus If latency is to be used to detect differences between normal and abnormal auditory mechanisms it is necessary to quantitatively discribe all latency parameters of the acoustic reflex The non linear portion of the response must be investigated as well as differences in relaxation of response as noted by Norris et al (1974)

Colletti (1974) has attempted to standardize the parameters of acoustic reflex response He defines several latency periods as described in

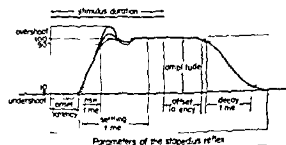


Fig 3 Definitions of acoustic reflex parameters as defined by Colletti

Fig. 3, and considers three parameters: onset latency, rise time, and amplitude to contain important biologic information. Colletti studied these latency parameters with varying intensities of step stimuli at four frequencies (500, 1000, 2000 and 4000 Hz). He reports that these three parameters are a function of stimulus intensity. The definitions are:

Onset latency:
The time interval between onset of stimulus and 5% of the maximum amplitude of the response.

Rise time:
The time required for the response to rise from 10 to 90% of its final value.

Amplitude:
Expressed in arbitrary units as width of its response at steady state.

Offset latency:
Time interval for the response to fall 95% of its value after cessation of the stimulus.

Decay time:
The time interval between 90 and 10% of the amplitude of the response after stimulus is over.

Strasser (1975) used a 1000 Hz acoustic stimulus to elicit acoustic reflexes in normal subjects and subjects with acoustic neuromas. He found that the subjects with acoustic neuromas exhibited initial latencies that were 30 msec longer than the mean latency value for the normal subjects. Latency in Strasser's study was defined as the period from signal onset to beginning of initial response.

Signal parameters and the acoustic reflex

The different parameters of the acoustic reflex are influenced by variations in stimulus parameters (e.g. intensity, duration, frequency, rise time). Djupesland and Zwislocki (1971) found stimulus duration had a definite effect on the reflex threshold. As stimulus duration increased, the intensity needed to elicit the acoustic reflex decreased. They attributed this occurrence to temporal summation occurring at or below the level of the superior olive complex in the acoustic reflex arc.

Woodford et al. (1975) have investigated the threshold of the reflex as a function of stimulus duration. Measuring clinical reflex thresholds, this group reported ART was dependent on duration of the stimulus. They found variations of as much as 30 dB over a 10–500 msec range of stimulus duration.

Sunderland (1974) used a 1000 Hz tone with varying rise times to elicit the acoustic reflex in a group of normal subjects. He found that the latency period increased as the signal rise time increased. They attributed this occurrence to temporal summation occurring at or below the level of the superior olive complex in the acoustic reflex arc.

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Effect of disease states on the acoustic reflex

The acoustic reflex is modified by various disease states at middle ear, cochlear, or retrocochlear portions of the peripheral auditory system. Presence of middle ear disease can prevent recording of acoustic reflex activity (Klockhoff, 1961; Jepsen, 1963; Feldman, 1970; Jerger, 1974). Jerger (1974) has shown that as little as a 5 dB air bone gap in the ear

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ıcr ı (Colletti 1975) *Consequently*, accu-
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acoustic reflex makes it possible to detect auditory nervous system abnormalities before changes in pure tone thresholds occur

Summary

The parameters of the acoustic reflex vary with age, disease state and characteristics of the eliciting acoustic stimulus Reflex threshold has been defined several ways Clinically, balance meter deflection is commonly used as an indicator of reflex activity for threshold determination In an effort to quantify the acoustic reflex threshold different researchers have proposed that values of 5 or 10% of maximal attained impedance change be defined as the threshold value Latency has been defined as the period from signal onset to 5 or 10% of maximum amplitude of the response, or as the period from signal onset to the beginning of impedance change Latency is contingent on the definition used for acoustic reflex threshold Review of the literature indicated acoustic reflex latency and threshold need to be more precisely defined Little clinical evidence is available to indicate the effect signal rise time has on reflex latency values

III. Experimental Procedure

Subjects

Eleven adults between the ages of 20–35 years with no history of chronic middle ear disease, vertigo, tinnitus or difficulty with Eustachian tube function were selected as subjects Audiometric pure tone thresholds for all subjects were measured using standard audiometric equipment calibrated to ISO, 1964 standards, and ASHA draft guidelines for audiometry (Wilson et al 1973) Bilateral thresholds had to be 20 dB HL or better for frequencies 250–4000 Hz Speech reception thresholds were within 5 dB of pure tone averages for 500, 1000 and 2000 Hz In addition, the subjects had normal tympanograms as described by Jerger (1974*a*) Tympanometry was done immediately prior to reflex recording using a Madsen ZO 72 impedance bridge and techniques described in the Madsen manual

Acoustic stimulus

In this investigation intensity frequency and signal duration of the acoustic stimulus were held constant The rise and fall time of the signal was the only stimulus parameter which was allowed to vary The acoustic signal was triggered manually and displayed simultaneously on an oscilloscope with the acoustic reflex

A 2000 Hz pure tone was generated and the frequency was set using an electronic counter

Intensity of the acoustic stimulus was amplified by a power amplifier and attenuated by two step attenuators A sound level meter and acoustic coupler was used at the beginning and end of data collection to measure the sound pressure level of the pure tone Pure tones were delivered to a PDR 600 earphone with an MX-41/AR cushion

The AR threshold was obtained by ascending measurements in 2 dB steps The threshold was defined as the level in dB, that produced a consistent two unit deflection on the balance meter of the Madsen electroacoustic impedance bridge The AR was elicited by presentation of the acoustic stimulus at 10 dB above the subject's clinical reflex threshold

Variation in rise/fall time of the acoustic signal was used to obtain quantitative data on acoustic reflex threshold and latency parameters An electronic switch controlled the rise and fall time Four different times were used fast,[1] 50 msec, 100 msec, and 250 msec Presentation of the rise/fall time of the acoustic signal was randomized to avoid any order effect The fast rise time served as a control signal and represented maximal response speed measurable with this equipment Under this condition, hysterisis was minimized and maximal effects of time constants were allowed The time and speed with which the meter of the Madsen bridge deflects is nonlinear and is based on the ability of the equipment to respond to the impedance change resulting from middle ear muscle contraction Latencies measured with the fast (i e , "control") stimuli are influenced by equipment time constants and represent measurements that identify these variables with the Madsen bridge The slower rise times (50, 100, 250 msec) provided information necessary for a quantitative definition of the acoustic reflex thresholds and latencies

[1] The stimulus reached full amplitude within 1/2 cycle

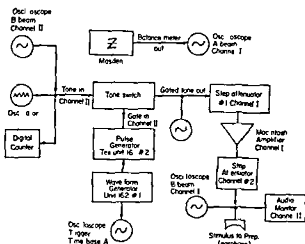


Fig 4 Block diagram of equipment

The acoustic signal had a duration of 600 msec A pulse generator and waveform generator controlled the duration for the pure tone Due to the characteristics of the switch, the fast signal was adjusted on the oscilloscope to a total duration of 600 msec. The other test signals with varying rise/fall times were triggered, rose to maximum amplitude, then terminated after 600 msec had elapsed The resulting effect was to have these signals at full amplitude for varying lengths of time The 50 msec signal was at full amplitude for 550 msec, the 100 msec signal at full amplitude for 500 msec, and the 250 msec signal at full amplitude msec The rise time of the signal and duration at full amplitude were carecified and controlled Since this study considering only the onset portion of the acoustic reflex, variations in total duration that occurred beyond the 600 msec period are not important for the purposes of this study

A sound level meter was used to calibrate the intensity of the acoustic signal The electracoustic impedance bridge was calibrated at the beginning of data collection with a 2 cm³ coupler to insure accuracy of the compliance scale

Recording of data

Tympanometry and acoustic reflex measurements were completed using the Madsen ZO 72 electroacoustic impedance bridge with a 220 Hz probe tone Testing techniques were the same as those described by Jerger (1974 *a*, *b*, *c*) and Habener and Snyder (1974) Tympanograms were recorded on a 7035B X–Y recorder The output from the Madsen bridge was connected to a dual beam oscilloscope The response of the balance meter output was amplified and displayed on the oscilloscope Both the reflex and the stimulus were photographed with an oscilloscope recording camera for later analysis A block diagram of the equipment is presented in Fig 4

Analysis of data

Data was collected in six test runs Five samples were taken at each signal rise time Data analysis was based on median values using a minimum of three to a maximum of five tracings per stimulus condition Oscilloscope displays were photographed and read from a microfilm reader Each acoustic signal and reflex response was traced from the microfilm reader Baseline was established by drawing a

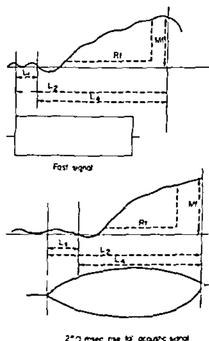


Fig 5 Definitions of acoustic reflex latency

line through the impedance trace produced by the phsysiological noise The point at which the impedance trace crossed baseline was de fined as threshold of the acoustic reflex The time course of the different reflex responses was measured from these tracings An exam ple is seen in Fig 5

Definition of terms

Fig 5 diagrammatically illustrates the follow ing definitions of latency

L_1 *(latency 1)* The time period from signal onset to beginning of impedance change was designated L_1 for the fast rise time and is the most rapid response time which can be meas ured with this equipment The L_1 value was subtracted from the initial latency period (i e the period from signal onset to beginning of impedance change) obtained with the other three signal rise/fall times (50 100 250 msec) This permits a partial control for the time con stant of the equipment However due to the of the Madsen ZO 72 impedance not possible to eliminate all constants from the analysis

L_2 *(latency 2)* This is defined as the period from signal onset to full amplitude of the acoustic reflex for all stimulus conditions

L_S *(latency S)* This is the time period from signal onset to the beginning of impedance change for signal rise time of 50 100 and 250 msec

L_3 *(latency 3)* This time period was calcu lated by substracting the L_1 value from L_S ($L_S-L_1=L_3$) and is the true latency of the acoustic reflex threshold

L_4 *(latency-4)* This is the time period from impedance change to full amplitude of the acoustic reflex

L_5 *(latency 5)* This is the time period from signal onset to maximum reflex amplitude corrected for the time constants of the equip ment ($L_3+L_4=L_5$)

Rise time of the acoustic reflex The rise time of the acoustic reflex is the time period required for the acoustic reflex response to rise from 0 to 10% of its final value

Mf maximum impedance change Ex pressed in arbitrary units this measurement represents the maximal amplitude of the reflex response measured from baseline to point of maximum impedance change

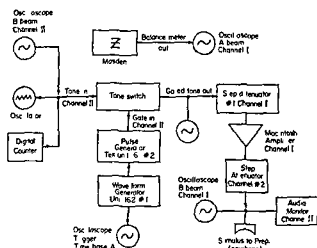


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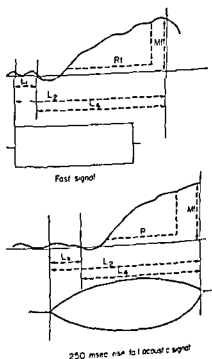


Fig 5 Definitions of acoustic reflex latency

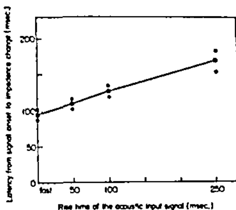


Fig 6 Latency from signal onset to impedance change as a function of rise time of the acoustic input signal

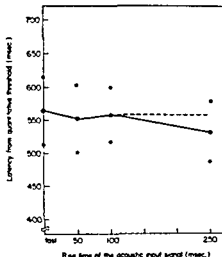


Fig 8 Latency from quantitative threshold as a function of rise time of acoustic input signal

change, to the point of maximum impedance change as a function of the rise time of the acoustic input signal This latency parameter appeared to remain constant indicating that once threshold is crossed, the time period necessary for the contraction to occur remains stable Again, the explanation for the lower mean latency value for the 250 msec rise time may be the equipment limitation previously discussed The full time course of the onset phase was not photographed for the 250 msec signal The dotted line on the graph represents a more accurate value for mean latency expected with the 250 msec rise time signal than the 532 msec measured mean value This latency period (L_4) is a direct measure of the ability of the contractile phase of the reflex The measurement isolates the contraction phase by eliminating equipment time constants and other external variables which could make a spurious contribution to reflex latency values

Fig 9 shows latency of threshold as a function of the rise time of the acoustic input signal The result is a linear function with latency

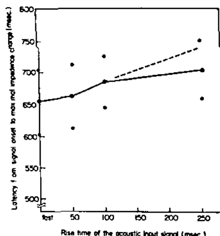


Fig 7 Latency from signal onset to maximum impedance change as a function of rise time of the acoustic input signal

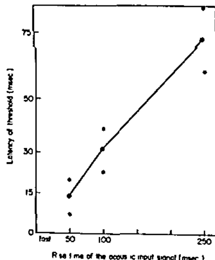


Fig 9 Latency of threshold as a function of rise time of the acoustic input signal

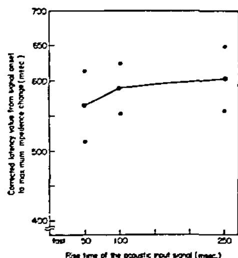


Fig 10 Latency from signal onset to maximum impedance change corrected for equipment time constants as a function of the acoustic input signal

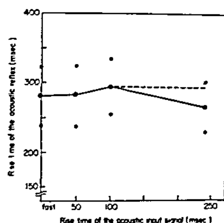


Fig 11 Rise time of the acoustic reflex as a function of the acoustic input signal

of threshold increasing as rise time of the acoustic input signal increases This result indicates that there is a critical intensity level which must be reached to trigger the acoustic reflex L_1 represents true latency of the acoustic reflex as recorded L_3 on the other hand represents the time period from stimulus onset to impedance change for acoustic signals of varying rise times By subtracting the L_1 latency values from the L_3 latency values the latency of the threshold can be obtained

Fig 10 displays the latency period from signal onset to maximum impedance change *after correction of time constants for the impedance bridge* This indicates that the latency period increases with increasing rise time of the acoustic stimulus This reflects the increased value of L_3 with increasing signal rise time As mentioned previously L_4 is artificially shortened for the 250 msec signal due to equipment limitations One would expect the L_3 value to be even longer than the 606 msec shown in Fig 10

Fig 11 shows the rise time of the acoustic reflex as a function of the rise time of the acoustic stimulus The rise time values of the acoustic reflex remain essentially constant at 280–290 msec for the fast 50 and 100 msec rise times and drops to 265 msec for the 250 msec rise time signal This reflects equipment limitations previously discussed, the full reflex response was not recorded for the 250 msec rise time signal

Fig 12 shows maximal impedance change as a function of rise time of the acoustic input signal It is evident from this figure that there is no change in the maximum impedance as a function of signal rise time

Statistical analysis of data

The initial latency parameter (L_S L_1) obtained with each rise time was subjected to a one way analysis of variance Error variance within each group was compared to the total error (variance) *between groups An F test* was conducted to determine the significance of the

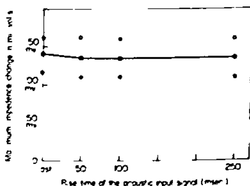


Fig 12 Maximum impedance change as a function of rise time of the acoustic input signal

Table IV *One way analysis of variance of L_1 and L_4 latency scores presented in Table II*

Source	SS	df	MS	F	Significance
Between groups	67 307 1	3	22 435 7	72 14	
Within groups	25 816 9	83	311 0		$P<0\ 001$
	93 124	86			

L_4 R_t and M_f scores show greater error within group than between groups therefore, any differences between groups is not significant

Table V *Comparison values between groups individual comparison among group means*

		Group		
Group	Mean	II 110	III 127 7	IV 170
I	96 0	−13 6	−31 3	−73 6
II	110		−17 7	−60
III	127 7			−42 3

[illegible] confidence

effect varying signal rise time had on the initial latency parameter The analysis of variance produced a significant ($P<001$) F ratio of 72 14 as seen in Table 4, suggesting a difference in the mean latencies However, it does not show where the difference occurred A comparison test was then employed The mean values for initial latency at each rise time were tested for significance at the 0 05 level of confidence Table V shows that significant differences were obtained between all but Group I versus Group II The signal with the 50 msec rise time did not produce a significant change in latency values Signals with longer rise times (100 msec and 250 msec) did elicit reflexes with significantly longer initial latency periods

A one-way analysis of variance showed there was no significant difference between L_4 (latency from impedance change to full amplitude of the acoustic reflex) values measured with any of the four different signals Variance analysis showed that rise time (R_t) and maximum impedance change (M_f) did not vary with different signal rise times The results shown in *Table II indicate the mean values for* these latency parameters varies within a narrow range for the four test signals Variance analysis indicated there was far greater variance within each group than existed between groups

A summary of the statistical analysis of the experimental data showed that initial latency values increased as acoustic signal rise time increased Acoustic signal rise times of 100 msec or longer produced significant changes in measured latency values Once threshold crossing occurred, however, signal rise time had no effect on latency parameters Values for L_4 R_t and Mf remained constant across the four signal conditions

V. Discussion

The purpose of this investigation was to define in quantitative terms acoustic reflex latency parameters and investigate the effect of acoustic signal rise time on these latency parameters in a normal hearing population. Acoustic reflex threshold in normal humans has been found to vary with stimulus duration, type of stimulus and age of subject. Latency is contingent on ART, thus, signal parameters which effect threshold will effect latency.

Moller (1974) has discussed the difficulty involved in quantifying the acoustic reflex. Based on investigations with both human and animal subjects (Borg, 1972, Møller, 1962), a value of 10 percent of the maximal obtainable impedance change of the acoustic reflex has been defined as threshold. This 10% value is considered a sensitivity measure of the acous- reflex. This study has defined quantitative threshold as the point of impedance change. Quantitative threshold is then used as a measuring point of determining the length of various latency parameters. It is believed that the definition of threshold as the point of impedance change is a more reliable method than the 10% criteria suggested by Moller. Use of quantitative threshold involves one less measurement, therefore, one less opportunity for error. In any of these acoustic reflex measurement procedures, a judgement must be made to establish baseline value. The point at which the reflex crosses the baseline is judged to be the quantitative threshold. In order to use Moller's 10% measure as threshold, it is necessary to establish baseline value, measure maximum amplitude, and then determine the point at which 10% of the maximum impedance change occurred. Using the point of impedance change as threshold would provide a specific point from which to measure latency regardless of whether the initial impedance change was in a positive or negative direction (an upward or downward deflection of the reflex tracing).

Determining the threshold by observation of balance meter deflection is quite sufficient for clinical work. Quantitative thresholds are necessary for more precise calculation of latency periods. Variables external to the physiological system itself cause a change in the initial period, that is, the latency period from signal onset to impedance change. Initial latency varies with equipment time constants, the strength, duration, and type of the acoustic stimulus, and the rise time of the acoustic stimulus.

Time constants of various electroacoustic impedance bridges have a bearing on latency values. The time constant determines the speed with which the bridge can respond and hence limits measurement capabilities. Thus, events occurring in a shorter time span than the time constants of the bridge cannot be measured. In this study the fast signal rise time was used to allow for minimal hysteresis but maximal effects of time constants. Time constants of the equipment must be considered if latency is measured from signal onset to beginning of physiological response.

Initial latency is most commonly defined from signal onset to beginning of the acoustic reflex response. Colletti (1975) used 5% of maximal impedance change and Møller (1974) used 10% of the maximal impedance change of the acoustic reflex response as measurement points. Strasser (1975) and Sunderland (1974)

Table VI

Rise time of stimulus	Measured latency value	
	McPherson & Thompson (2 000 Hz)	Sunderland (1974) (1 000 Hz)
Fast	96 4 msec	103 msec
50 msec	110 0 msec	125 0 msec
100 msec	127 0 msec	148 0 msec
250 msec	170 0 msec	242 0 msec

measured initial latency from signal onset to beginning of impedance change Irrespective of the definition, investigators have shown *that this latency period is dependent on signal* intensity Specifically, as intensity increases, latency decreases (Dallos, 1964)

One purpose of this study was to examine the effect of signal rise time on latency Holding intensity constant, the initial latency period was varied with the signal rise time Results displayed in Fig 1 demonstrated a linear relationship with an increase in latency with increasing rise/fall time of the acoustic signal This data is in agreement with Sunderland (1974) (see Table VI)

Latency should be measured from the point of impedance change rather than from the signal onset Measuring latency in this manner eliminates the initial latency (L_i or L_S) and thus avoids the need to consider equipment time constants, or rise time of the acoustic stimulus in calculation of latency values This provides a more stable latency measure

Latency values measured by Sunderland (1974), and in this study, show that latency increases as signal rise time increases However, the absolute values differ There are two possible explanations for the differing numerical values 1) differences in the frequencies of the acoustic stimulus, and 2) differences in the recording methods used Sunderland used a 1 000 Hz acoustic stimulus and a polygraph to record acoustic reflex response This study used a 2 000 Hz acoustic stimuli and an oscilloscope to record reflex activity However, it is felt that the differences in frequencies are the contributing factor

If the intention is to measure the initial latency parameter, it is imperative to control and specify all physical parameters of the acoustic signal used to elicit the reflex Physical characteristics of the signal, both intensity and rise time, cause a change in energy of the stimulus envelope and hence in initial latency values Calibration of the acoustic stimulus with specification of intensity, duration and rise time is therefore necessary This initial latency should be measured from signal onset to quantitative threshold, the point of impedance change

One approach to the variations found in the initial latency period considers the time period required to generate sufficient acoustic energy to reach threshold An interesting question is, given sufficient acoustic energy to reach threshold, how long does it then take the acoustic reflex to respond? The L_i latency is the actual latency of the reflex while the difference of L_S and L_i is the true latency of the acoustic reflex threshold

The acoustic reflex, being a suprathreshold phenomenon, appears to act as an energy related response attuned to basilar membrane response in the cochlea Amplitude and vertical velocity of membrane displacement are proportional to stimulus intensity The rise time of the acoustic stimulus determines the speed at which the basilar membrane reaches its amplitude and velocity maxima Rise time controls the energy available in the acoustic stimuli envelope at any given instant Since these phenomena occur at suprathreshold levels, continuing change in basilar membrane velocity will occur until the energy envelope of the rise time reaches a "threshold level and the acoustic reflex response occurs

Møller (1974) has stated that the acoustic reflex functions as a lowpass filter, the input is the envelope of the sound and the output is the muscle response Varying the rise/fall times of the acoustic stimuli provided a method for studying the envelope of the sound Changes

in the sound envelope did have an effect on the initial latency However the effect of this envelope was to determine the time necessary to cross threshold Once threshold was crossed the shape of the sound envelope had no effect on the muscle response That is rise time maximum impedance change of the acoustic reflex and the L_4 (latency from quantitative threshold to maximal impedance change) was not altered by variations in signal rise times

Fig 11 illustrates that rise time of the acoustic reflex remains constant at three signal rise times fast 50 and 100 msec The dotted line on the graph is a more accurate representation of reflex rise time for the 250 msec rise/fall acoustic signal The 250 msec signal has less total acoustic energy present in 600 msec than the fast signal If a change in rise time occurred one would expect longer rather than shorter rise time values The rise times measured were from 200 to 375 msec for this group of normal subjects Actual total acoustic energy in the signal is the same Fig 11 demonstrates that acoustic reflex rise time remains constant across the signal conditions This indicates that the energy variation between the four signals was insignificant that is although the total energy contained in the stimulus envelope differed the reflex did not demonstrate knowledge of the slope of the envelope but only of its most recent state (the amount of energy occurring at that point in time)

The action of the acoustic reflex may be viewed as an energy related phenomena If the acoustic reflex behaves as a threshold phenomena then when ART (a specific intensity threshold) is reached the acoustic reflex would fire regardless of the total energy received However if the energy principle is operative then latency would change and be related to the total energy under the curve before the reflex occurs Considering the energy principle as a reasonable hypothesis there is a finite time that the reflex is sampling the incoming signal Storage of intensity information would occur for a discrete finite time When the sampling procedure recognizes that enough energy is present then the acoustic reflex would occur Accepting the energy phenomena hypothesis would provide an explanation for a mechanism of the phenomena of loudness recruitment It is possible that a recruiting auditory system stores energy for a longer time period than does a non recruiting system At a specific point the sum of this stored energy overloads the nerve triggering the acoustic reflex at sensation levels much lower than those occurring in the normal system The storage mechanism is presumed to occur in the sensory receptor organ the hair cell The behavior of the reflex with acoustic stimuli of varying rise times would lend support to the energy hypothesis although not provide conclusive evidence All acoustic stimuli were suprathreshold of sufficient intensity to trigger the reflex yet once threshold crossing occurred the shape of the sound envelope had no effect on the rise time of the reflex the maximal impedance change or L_4 latency parameters The strength of the response was determined by the energy level at which the acoustic reflex fired Latency was related to the total energy in the stimulus envelope variation in the stimulus envelope effected only the length of the initial latency parameter It appears that latency is dependent on the slope of the envelope but once threshold is crossed the amount of energy last measured determines the remaining reflex parameters The last energy point sampled bears on the response of L_4 rise time of the acoustic reflex and maximal impedance change The fact that these parameters remained constant indicates that the threshold point energy remained constant The shape of the stimulus envelope effects the length of the latency from signal onset to reflex response because envelope shape determines the amount of energy available for sampling at any given instant

VI. Summary and Conclusions

This study defined and quantified reflex latencies in humans with normal auditory function. Furthermore, it examined the stimulus rise time in relation to changes in the acoustic reflex latency parameters.

A carefully controlled and monitored 2000 Hz acoustic signal with four different rise times was presented 10 dB above each subject's clinical reflex threshold. The acoustic reflex response was photographed from an oscilloscope and read from a microfilm reader. The time course of the reflex responses was measured. Mean values for the latency parameters, maximum impedance changes and acoustic reflex rise times were determined.

It has been proposed that latency be defined from the point of impedance change (quantitative reflex threshold) rather than from signal onset. The purpose of this definition is to provide a less variable latency measure. This latency would be expected to vary with intensity of the acoustic stimulus within the dynamic range of the reflex. However, it would not be affected by other variables such as signal rise time, signal duration, or time constants of the electroacoustic impedance bridge.

Initial latency of the acoustic reflex is defined from signal onset to beginning of impedance change with all characteristics of the acoustic stimuli specified. The length of this initial latency varies as a function of stimulus rise time and appears to be energy dependent. Comparisons of latency values obtained with various impedance bridges should not be made until delay factors inherent in the equipment are more precisely specified.

Latency from quantitative threshold to maximal impedance change (L_4), rise time, and maximal impedance change of the acoustic reflex are not effected by variation in rise time of the eliciting stimulus. Quantitative values found in this study for different parameters of the acoustic reflex are as follows: L_1, 96.4 msec, L_4, 565.5 msec, and rise time of the acoustic reflex (R_t), 281.6 msec.

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Appendix A

Median values (in msec) for latencies, rise time of the acoustic reflex and maximum impedance change (in mv) listed by subject

Acoustic signal Fast rise/fall

Subject	L_1	L_2	L_4	R_t	MI
1	100 msec	710 msec	600 msec	260 msec	155 mv
	100	720	620	310	140
2	95	690	610	290	130
	95	625	535	290	175
3	85	725	640	270	155
	90	740	650	425	145
4	90	670	580	280	145
	90	600	510	370	90
5	95	700	620	240	120
	95	635	545	300	145
6	100	705	605	285	143
	90	660	565	310	115
7	100	455	440	80	240
	90	280	180	90	143
8	100	705	615	310	123
	90	750	660	440	120
9	100	635	535	230	240
	110	590	495	245	200
10	100	705	620	335	60
	100	720	610	310	100
11	105	715	605	305	115
	100	680	600	220	110
mean	96 4	655 2	565 5	281 6	141 32
s d	6 0	106 3	101 7	83 97	43 2

Acoustic signal 50 msec rise/fall

Subject	L_s	L_2	L_3	L_4	L_5	R_t	Mf
1	115	750	15	635	650	395	140
	100	680	0	580	560	330	150
2	100	650	5	550	555	350	123
3	110	720	25	610	635	340	135
	90	710	0	620	620	400	105
4	90	530	0	440	440	220	125
	125	685	35	560	580	360	93
5	100	685	5	585	590	275	135
	105	675	10	570	590	290	160
6	140	730	40	590	630	260	170
	80	530	-10	450	450	240	100
7	100	610	0	510	510	140	240
	105	280	15	175	190	110	155
8	130	660	30	530	565	305	115
	130	770	40	640	680	420	140
9	110	720	10	610	620	280	260
	130	710	20	580	600	170	210
10	110	715	10	605	615	355	68
	120	700	20	580	590	250	90
11	100	720	-5	620	625	270	110
	120	670	20	550	570	180	45
mean	110 0	661 9	13 6	551 9	565 5	282 9	136 6
s d	15 5	106 8	13 6	101 5	104 3	86 0	52 2

Acoustic signal 100 msec rise/fall

Subject	L_s	L_2	L_3	L_4	L_5	R_t	Mf
1	125	745	25	620	640	360	145
	120	770	20	650	670	260	180
2	130	650	35	520	555	320	130
	140	690	45	550	605	400	143
3	130	740	45	610	650	310	125
	120	740	30	620	650	380	120
4	120	560	30	440	470	230	145
	110	710	20	600	610	320	90
5	110	730	15	620	635	270	135
	130	740	35	610	650	335	135
6	120	740	20	620	635	370	133
	140	530	50	390	445	270	75
7	110	640	10	530	530	200	240
	120	470	30	350	460	100	105
8	100	750	0	650	655	380	130
	160	770	70	610	680	420	165
9	165	675	65	510	575	295	205
	140	665	30	525	540	160	190
10	150	670	50	520	570	260	60
	120	640	20	520	520	270	95
11	120	720	15	600	605	350	115
	120	720	20	600	640	220	45
mean	127 7	684 8	30 9	557 5	590 5	294 6	132 1
s d	16 3	79 1	17 4	81 6	70 6	80 6	46 1

Acoustic signal 250 msec rise/fall

Subject	L_s	L_2	L_3	L_4	L_5	R_t	' f
1	120	740	20	620	640	270	145
	140	650	40	510	550	280	153
2	200	700	105	500	605	260	120
	150	645	55	495	555	230	150
3	180	810	95	630	715	330	142
	135	790	45	655	700	300	90
4	165	605	75	440	500	200	130
	160	630	70	470	580	340	95
5	150	750	55	600	655	260	130
	160	770	65	610	655	340	150
6	190	780	90	590	660	300	120
	150	740	60	590	660	310	90
7	150	470	50	320	370	100	190
	190	630	100	440	535	210	100
8	180	770	80	590	670	400	125
	210	810	120	600	710	340	145
9	190	690	90	500	600	230	265
	230	530	120	300	410	150	210
10	170	720	70	550	630	270	75
	160	750	60	590	650	270	95
11	180	740	75	560	635	210	165
	180	730	80	550	650	250	40
mean	170	702 3	73 6	532 3	606 1	265 9	132 95
s d	26 1	88 8	25 5	94 2	89 9	68 2	48 3

Appendix B (raw data)

Fast rise time data

Subject #1	L_1	L_2	L_4	R_t	Mf	Subject #2	L_1	L_2	L_4	R_t	Mf	Subject #3	L_1	L_2	L_4	R_t	Mf	Subject #4	L_1	L_2	L_4	R_t	Mf
Sample 1 (R)	110	710	600	260	155		80	690	610	270	120		90	730	640	250	150		100	700	600	280	145
(L)	100	730	630	310	160		80	610	530	290	185		90	730	640	320	110		80	680	600	440	85
Sample 2 (R)	10	[illegible]	580	10	165		100	680	580	340	130		80	720	6 0	270	170		90	670	580	360	150
(L)			0	50	160		90	480	390	220	170		80	[illegible]	640	430	140		90	600	510	300	90
[illegible] (R)		0	620	360	150		100	740	6 0	290	170		100	740	640	270	160		80	560	480	280	145
(L)	60	730	650	360	140		100	700	600	320	180		90	750	660	440	150		100	600	500	370	100
[illegible] (R)			-	-	-		95	-	-	-	-		80	680	600	280	150		-	-	-	-	-
(L)	110	710	600	380	130		100	640	540	290	150		90	750	660	420	170		-	-	-	-	-
Sample 5 (R)		-	-	-	-		90	-	-	-	-		-	-	-	-	-		-	-	-	-	-
(L)	100	600	500	700	120		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Median (R)	100	710	600	260	155		95	690	610	290	130		85	725	640	270	155		90	670	580	280	145
Value (L)	100	[illegible]	670	310	140		95	625	535	290	175		90	740	650	425	145		90	600	510	370	90

Subject #5	L_1	L_2	L_4	R_t	Mf	Subject #6	L_1	L_2	L_4	R_t	Mf	Subject #7	L_1	L_2	L_4	R_t	Mf	Subject #8	L_1	L_2	L_4	R_t	Mf
Sample 1 (R)	80	700	670	240	120		120	740	620	310	140		100	640	540	180	270		100	720	620	310	125
(L)	90	690	600	310	140		100	640	540	360	120		90	260	170	80	155		90	720	630	400	120
Sample 2 (R)	90	730	640	320	125		110	700	590	260	155		90	290	200	80	280		100	700	600	310	120
(L)	110	620	510	300	165		90	690	600	220	105		90	300	210	90	130		100	780	680	580	135
Sample 3 (R)	90	670	380	240	115		90	710	620	310	140		100	310	210	70	240		70	700	620	240	115
(L)	100	650	550	280	150		90	680	590	340	130		90	280	190	120	140		90	750	660	440	100
Sample 4 (R)	-	-	-	-	-		80	660	580	240	145		80	600	520	70	240		100	710	610	460	130
(L)	80	620	540	300	120		80	490	410	280	110		100	250	150	90	145		-	-	-	-	-
Sample 5 (R)	-	-	-	-	-		-	-	-	-	-		100	240	440	220	200		-	-	-	-	-
(L)	-	-	-	-	-		-	-	-	-	-		-	-	-	-	-		-	-	-	-	-
Median (R)	95	700	620	240	120		100	705	605	285	143		100	455	440	80	240		100	705	615	310	123
Value (L)	95	635	565	300	145		90	660	565	310	115		90	280	180	90	143		90	750	660	440	120

Subject #9		L_1	L_2	L_4	R_t	Mf	#10	L_1	L_2	L_4	R_t	Mf	#11	L_1	L_2	L_4	R_t	Mf
Sample 1	(R)	100	600	500	230	240		60	700	640	320	60		110	700	590	310	115
	(L)	120	660	540	250	200		110	690	580	240	120		80	680	600	220	45
Sample 2	(R)	110	600	490	230	240		100	730	630	310	60		120	720	600	300	115
	(L)	120	560	440	240	200		100	730	630	310	115		100	490	390	160	110
ample 3	(R)	100	670	570	260	250		110	680	570	350	55		100	700	600	310	90
	(L)	100	620	620	250	200		100	710	610	310	100		100	710	610	300	110
imple 4	(R)	100	690	590	190	180		100	710	610	440	60		120	730	610	270	105
	(L)	80	530	450	170	180		120	720	600	340	100		-	-	-	-	-
imple 5	(R)	-	-	-	-	-		-	-	-	-	-		100	740	640	400	-
	(L)	-	-	-	-	-		80	720	640	310	90		-	-	-	-	-
dian	(R)	100	635	535	230	240		100	705	620	335	60		105	715	605	305	115
lue	(L)	110	590	495	245	200		100	720	610	310	100		100	680	600	220	110

50 msec rise time signal

Subject #1	L_m	L_3	L_4	L_5	R_t	Mf	Subject #2	L_m	L_3	L_4	L_5	R_t	Mf	Subject #3	L_m	L_3	L_4	L_5	R_t	Mf
Sample 1 (R)	120	20	600	670	290	135		100	5	560	565	300	130		140	55	610	665	410	125
(L)	100	0	590	580	310	160		-	-	-	-	-	-		80	-10	640	640	370	100
Sample 2 (R)	170	20	640	680	380	145		100	5	540	545	400	115		110	25	610	635	340	135
(L)	130	30	530	560	330	150		-	-	-	-	-	-		90	0	620	620	410	110
Sample 3 (R)	110	10	630	640	410	135		-	-	-	-	-	-		120	35	580	615	360	145
(L)	90	10	660	660	360	145		-	-	-	-	-	-		90	0	620	620	400	130
Sample 4 (R)	110	10	660	670	430	145		-	-	-	-	-	-		110	25	620	645	320	145
(L)		-		-	-	-		-	-	-	-	-	-		100	10	600	610	410	95
Sample 5 (R)	-	-	-	-	-	-		-	-	-	-	-	-		110	25	570	595	180	120
(L)	-	-	-		-	-		-	-	-	-	-	-		80	-10	680	680	400	105
Median Value (R)	115	15	635	650	395	140		100	5	550	555	350	123		110	25	610	635	340	135
(L)	100	0	590	560	330	150		-	-	-	-	-	-		90	0	620	620	400	105

Subject #4	L_m	L_3	L_4	L_5	R_t	Mf	Subject #5	L_m	L_3	L_4	L_5	R_t	Mf	Subject #6	L_m	L_3	L_4	L_5	R_t	Mf
Sample 1 (R)	90	0	440	440	240	130		100	5	580	585	300	135		140	40	590	630	340	160
(L)	130	40	540	580	320	95		110	15	560	575	270	165		80	-10	450	450	380	100
Sample 2 (R)	100	10	420	430	220	125		120	25	560	585	270	140		140	40	590	630	260	175
(L)	90	0	580	580	320	100		100	5	560	565	310	170		70	-20	450	450	240	90
Sample 3 (R)	90	0	570	570	220	105		100	5	590	595	280	135		130	-30	620	650	260	170
(L)	120	30	580	610	400	90		120	25	580	605	260	155		80	-10	650	650	120	135
Sample 4 (R)	-	-	-	-	-	-		100	5	620	625	260	125		-	-	-	-	-	-
(L)	130	40	540	580	460	90		100	5	610	615	330	115		-	-	-	-	-	-
Sample 5 (R)	-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
(L)	-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
Median Value (R)	90	0	440	440	220	125		100	5	585	590	275	135		140	40	590	630	260	170
(L)	125	15	570	580	360	93		105	10	570	590	290	160		80	-10	450	450	240	100

Subject #7		L_s	L_3	L_4	L_5	R_t	MF	Subject #9	L_s	L_3	L_4	L_5	R_t	MF	S bject #)	L_s	L_3	L_4	L_5	R_t	MF
Sample 1	(R)	110	10	600	610	270	280		140	40	530	570	380	110		110	10	650	660	400	250
	L)	110	20	170	190	1 0	160		1 0	30	650	680	4 0	120		130	0	80	600	150	210
Sample	R)	00	0	660	660	10	280		130	30	530	560	310	1 0		110	10	610	6 0	280	265
		00	0	80	90	70	150		130	40	6 0	6f0	4 0	140		1 0	10	590	600	50	2 0
Sample 3	R	00	0	5 0	5 0	110	240		1 0	0	450	4 0	240	110		93	10	6 0	6 0	100	275
		0	30	0	170	70	175		140	50	6 0	630	4 0	140		130	0	5 0	5 0	1 0	209
Sa ple 4	R)	110	10	1 0	350	80	30		130	30	6 0	6 0	300	170		1 0	50	570	620	50	210
	L)	90	0	190	190	80	145														-
Sample 5	(R)	100	0	300	300	140	220									100	0	580	550	230	60
	(L)	105	15																		
Median	(R)	100	0	510	510	140	40		130	30	530	565	305	115		110	10	610	6 0	280	260
Val e	(L)	105	15	1 5	190	110	155		130	40	640	680	4 0	140		130	0	580	600	170	10

Subject #10		L_s	L_3	L_4	L_5	R_t	f	S bje t #11	L_s	L_3	L_4	L_5	R_t	MF
Sample 1	(R)	110	10	670	630	00	60		100	5	570	570	330	110
	(L)	110	10	580	590	300	90		130	30	450	490	180	0
Sample	(R)	110	10	550	560	310	70		100	5	630	630	30	110
	(L)	1 0	0	580	600	310	100		100	0	600	600	00	40
Sample 3	(R)	100	0	630	630	370	85		100	5	6 0	6 0	210	115
	(L)	1 0	0	6 0	640	50	80		1 0	0	390	410	140	45
Sample 4	(R)	110	10	590	600	3 0	65		110	5	620	6 5	270	100
	(L)	1 0	0	560	590	150	90		1 0	0	550	570	180	105
Sample 5	(R)					-			90	15	650	630	320	160
	(L)	110	10	480	500	240	100		100	0	6 0	640	230	105
Median	(R)	110	10	605	615	355	68		100	5	6 0	625	270	110
Val e	(L)	1 0	20	580	590	250	90		1 0	0	550	570	180	45

10 msec rise time signal

Subject #1	L_8	L_3	L_4	L_5	R_t	Mf	Subject #2 L_8	L_3	L_4	L_5	R_t	Mf	Subject #3 L_8	L_3	L_4	L_5	R_t	Mf
Sample 1 (R)	1 0	70	6 0	6 0	60	1 5	130	35	460	495	7 0	170	170	35	600	635	2 0	90
(L)	1 0	0	650	6 0	3 0	190	1 0	45	600	6-5	520	1-5	140	50	610	660	390	90
Sample 2 (R)	1 0	0	590	6 0	360	1-5	170	35	570	555	370	150	1-0	55	590	635	760	130
(L)	1 0	0	6 0	660	760	150	140	45	460	505	350	125	170	30	670	650	-10	110
Sample 3 (R)	130	30	6 0	650	750	1-0	110	15	560	575	380	140	140	55	670	675	350	170
(L)	1 0	0	6 0	6 0	730	165	160	65	500	565	310	1-0	90	30	660	660	380	130
x					-	-	1 0	25	570	5-5	370	170	120	35	630	665	3-0	1 0
	1 0	0			-	-	1 0	45	610	655	-50	145	80	-10	600	600	-70	170
Sample 5 (R)		-	-	-	-	-	140	45	530	575	310	130	-	-	-	-	-	-
(L)	100	0		-	-	-	-	-	-	-		-	120	30	620	650	360	210
Median Value (R)	1 5	25	6 0	6-0	360	1-5	130	35	5 0	555	370	130	130	45	610	650	310	175
(L)	1 0	0	6 0	670	760	130	1-0	-5	550	605	400	1-3	120	30	670	650	390	120

Subject #4	L_8	L_3	L_4	L_5	R_t	Mf	Subject #5 L_8	L_3	L_4	L_5	R_t	Mf	Subject #6 L_8	L_3	L_4	L_5	R_t	Mf
Sample 1 (R)	1 0	30	600	-30	240	1 0	110	15	670	635	270	135	110	10	670	630	300	135
(L)	1 0	0	510	550	250	90	150	55	600	655	360	130	150	60	5 0	550	280	90
Sample 2 (R)	1 0	30	00	-30	210	1-5	110	15	5 0	555	200	170	120	70	670	6-0	4-0	130
(L)	110	0	610	630	3 0	90	130	35	6 0	655	360	1-0	140	50	3 0	390	180	70
Sample 3 (R)	100	10	500	510	230	140	100	5	650	655	310	1-5	150	50	-	-	-	-
(L)	100	10	600	610	3 0	90	1 0	75	670	6-5	310	130	150	60	-40	500	260	70
Sample 4 (R)	1 0	30	80	510	230	1-5	-	-	-	-	-	-	-	-	-	-	-	-
(L)	-	-	-	-	-	-	130	35	580	615	290	110	135	45	-		-	-
Sample 5 (R)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(L)	-	-	-	-	-	-	-	-	-	-	-	-	1-0	50	300	350	280	80
Median Value (R)	170	30	4-0	-0	230	1-5	110	15	670	635	270	135	170	70	670	635	1 0	133
(L)	110	0	600	610	370	90	130	35	610	650	335	135	140	50	390	-5	270	75

Subject #7		L_s	L_3	L_4	L_5	R_t	Mf	Subject #8	L_s	L_3	L_4	L_5	R_t	Mf	Subject #9	L_s	L_3	L_4	L_5	R_t	Mf
Sample 1	(R)	10	10	190	200	80	220		90	10	6 0	610	310	170		1 0	0	440	500	260	190
	(L)	110	0	610	630	100	100		160	0	610	680	360	170		1 0	30	5 0	570	160	00
Sample 2	(R)	100	0	590	580	210	2 0		110	10	660	670	50	135		160	60	5 0	600	310	190
	(L)	0	30	70	00	90	1 5		1 0	50	610	660	0	165		140	30	00	410	160	1 0
Sample 3	(R)	00	0	530	530	0	60							-		190	90	)	5 0	50	270
	(L)	1 0	30	510	540	110	105		160	70	660	710	4 0	150		100	10	510	510	140	1 0
Sample 4	(R)	130	30	60	490	110	210									170	0	5 0	6 0	300	7 0
	(L)	130	0	80	0	100	1 5									1 0	0	5 0	600	0	190
Sample 5	(R)	130	30	530	560	200	270										-				-
	(L)	100	10	350	460	1 0	100														-
Median Value	(R)	10	10	530	530	200	240		100	0	6 0	655	180	130		165	65	510	575	295	05
	(L)	1 0	30	150	0	100	105		160	0	610	680	4 0	165		140	30	5	5 0	160	110

Subject #10		L_s	L_3	L	L_5	R_t	Mf	Subject #11	s	L_3	L	L_5	R_t	Mf
Sample 1	(R)	50	50	50	500	2 0	65		130	5	650	675	3 0	115
	(L)	1 0	0	6 0	6 0	350	100		140	0	6 0	6 0	0	55
Sample 2	(R)	160	60	6 0	690	3 0	60		110	5	600	605	350	155
	(L)	110	10	610	6 0	70	95		130	30	600	690	10	90
Sample 3	(R)	150	50	5 0	570	260	60		130	75	460	485	140	110
	(L)	1 0	20	450	470	200	80		1 0	0	600	6 0	300	35
Sample 4	(R)	110	10	360	370	270	70		1 0	15	5 0	555	310	105
	(L)	150	50	0	490	280	00		1 0	0	670	640	240	45
Sample 5	(R)	1 0	0	590	610	240	55		100	-5	670	630	4 0	160
	(L)	100	0	5 0	520	220	90		1 0	0	5 0	570	2 0	45
Median Value	(R)	150	50	520	570	60	60		1 6	15	600	605	300	115
	(L)	1 0	0	5 0	5 0	270	95		1 0	20	600	6 0	2 0	45

250 msec rise time signal

Subject #1		L_a	L_3	L_4	L_5	R_t	Yf
Sample 1	(R)	120	20	630	650	310	140
	(L)	140	40	500	540	300	150
Sample 2	(R)	140	40	640	680	270	150
	(L)	140	40	410	470	280	155
Sample 3	(R)	170	20	610	670	270	150
	(L)	150	50	510	510	240	145
Sample 4	(R)	110	60	540	600	270	140
	(L)	140	40	600	640	300	145
Sample 5	(R)	-	-	510	-	-	-
	(L)	160	60	540	600	210	160
Median Value	(R)	120	20	620	640	270	145
	(L)	140	40	510	550	280	153

Subject #2		L_a	L_3	L_4	L_5	R_t	Yf
Sample 1	(R)	220	125	480	605	240	135
	(L)	140	45	530	575	250	150
Sample 2	(R)	140	45	530	575	280	115
	(L)	170	75	480	555	240	150
Sample 3	(R)	200	105	510	615	210	140
	(L)	140	45	510	555	210	160
Sample 4	(R)	220	125	500	625	260	120
	(L)	160	65	450	515	220	150
Sample 5	(R)	200	105	480	585	210	120
	(L)	-	-	-	-	-	-
Median Value	(R)	200	105	500	605	260	120
	(L)	150	55	495	555	230	150

Subject #3		L_a	L_3	L_4	L_5	R_t	Yf
Sample 1	(R)	180	95	600	695	260	130
	(L)	130	40	560	600	270	80
Sample 2	(R)	200	115	660	775	430	135
	(L)	140	50	670	720	360	130
Sample 3	(R)	240	55	680	735	360	150
	(L)	130	40	640	680	300	100
Sample 4	(R)	180	95	570	665	300	150
	(L)	140	50	680	730	300	70
Sample 5	(R)	-	-	-	-	-	-
	(L)	-	-	-	-	-	-
Median Value	(R)	180	95	630	715	330	142
	(L)	135	45	655	700	300	90

Subject #4		L_a	L_3	L_4	L_5	R_t	Yf
Sample 1	(R)	120	80	410	340	240	130
	(L)	270	130	400	600	400	95
Sample 2	(R)	160	90	420	510	200	130
	(L)	130	40	420	460	270	100
Sample 3	(R)	120	30	460	490	200	145
	(L)	160	70	510	580	340	90
Sample 4	(R)	160	70	360	430	170	155
	(L)	-	-	-	-	-	-
Sample 5	(R)	-	-	-	-	-	-
	(L)	-	-	-	-	-	-
Median Value	(R)	165	75	440	500	200	130
	(L)	160	70	470	580	340	95

Subject #5		L_a	L_3	L_4	L_5	R_t	Yf
Sample 1	(R)	120	25	670	695	300	125
	(L)	140	45	610	655	260	165
Sample 2	(R)	150	55	600	655	290	135
	(L)	150	55	600	655	340	150
Sample 3	(R)	150	55	600	655	250	140
	(L)	190	95	530	625	310	150
Sample 4	(R)	120	25	630	655	260	125
	(L)	200	105	660	765	520	100
Sample 5	(R)	150	55	600	655	220	130
	(L)	160	65	620	685	340	220
Median Value	(R)	150	55	600	655	260	130
	(L)	160	65	610	655	340	150

Subject #6		L_a	L_3	L_4	L_5	R_t	Yf
Sample 1	(R)	190	90	620	710	300	140
	(L)	160	70	590	660	310	90
Sample 2	(R)	160	60	600	660	350	150
	(L)	160	70	440	510	200	80
Sample 3	(R)	210	110	470	580	240	120
	(L)	140	50	620	670	340	95
Sample 4	(R)	220	120	590	710	330	105
	(L)	150	60	-	-	-	-
Sample 5	(R)	140	40	560	600	240	110
	(L)	150	60	-	-	-	-
Median Value	(R)	190	90	590	660	300	120
	(L)	150	60	590	660	310	90

Subject #7		L_s	L_3	L_4	L_5	R_t	Mf	Subject #8	L_s	L_3	L_4	L_5	R_t	Mf	Subject #9	L_s	L_3	L_4	L_5	R_t	Mf
Sample 1	(R)	150	50	320	270	100	190		220	120	500	620	400	115		180	80	500	580	190	260
	(L)	190	100	430	530	190	80		140	50	660	710	420	140		230	120	440	560	170	210
Sample 2	(R)	150	50	620	670	90	170		130	30	680	710	430	140		200	100	470	570	210	280
	(L)	150	60	450	510	230	120		210	120	600	720	340	160		220	110	300	410	130	210
Sample 3	(R)	140	40	270	260	80	220		180	80	590	670	330	125		180	80	520	600	190	265
	(L)	190	100	560	660	250	110		220	130	570	700	340	145		240	130	280	410	150	200
Sample 4	(R)	150	50	320	370	110	220		-	-	-	-	-	-		190	90	550	640	330	230
	(L)	210	120	420	540	180	90		-	-	-	-	-	-		-	-	-	-	-	-
Sample 5	(R)	160	60	410	470	160	190		-	-	-	-	-	-		230	130	480	610	300	240
	(L)	-	-	-	-	-	-		-	-	-	-	-	-		-	-	-	-	-	-
Median Value	(R)	150	50	320	370	100	190		180	80	590	670	400	125		190	90	500	600	230	265
	(L)	190	100	440	535	210	100		210	170	600	710	340	145		230	120	300	410	150	210

Subject #10		L_s	L_3	L_4	L_5	R_t	Mf	Subject #11	L_s	L_3	L_4	L_5	R_t	Mf
Sample 1	(R)	160	60	600	660	270	75		130	25	650	675	390	115
	(L)	140	40	520	560	240	90		140	40	620	660	200	55
Sample 2	(R)	170	70	500	570	280	70		110	5	600	605	350	115
	(L)	160	60	590	650	260	95		130	30	600	690	200	90
Sample 3	(R)	240	140	560	700	240	65		130	25	460	485	140	110
	(L)	140	40	480	520	270	95		120	20	600	670	300	35
Sample 4	(R)	210	110	520	630	230	85		120	15	540	555	310	105
	(L)	170	70	610	680	310	100		120	20	620	640	240	45
Sample 5	(R)	140	40	550	590	390	85		100	-5	630	630	420	160
	(L)	170	70	600	670	300	110		120	20	540	560	220	45
Median Value	(R)	170	70	550	630	270	75		120	15	600	605	350	115
	(L)	160	60	590	650	270	95		120	20	600	640	220	45

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SUPPLEMENT 354

Light and Electron Microscopic Studies on Material obtained by Fine Needle Biopsy

A methodological study on aspirates from tumours of the head and neck region with special emphasis on salivary gland tumours

BY

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STOCKHOLM, SWEDEN

From the Department of Oto-Laryngology (Head H Engström) and the Institute of Pathology Department of Clinical Cytology (Head B Stenkvist) University of Uppsala

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A methodological study on aspirates from tumours of the head and neck region with special emphasis on salivary gland tumours

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EVY HAGELQVIST

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Department of Cl nı [illegible] nkvist) University of [illegible]

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INTRODUCTION

Material obtained by fine needle aspiration biopsy has been used in Sweden for many years in the diagnosis of tumours and has become a common tool in clinical practice The pioneers in the development of this technique were Söderström (1952 1966) and Franzen (1960 1968) but many other authors (Zajiceck, 1965, 1970 1974, Esposti et al 1968) followed suit and numerous works on methodology and results have been published All these studies are based on light microscopic observations During the same period the development of transmission electron microscopy (TEM) prompted several pathologists to study sectioned material from tumours in the electron microscope and this literature is rapidly increasing (Mori & Lennert 1969 Murad 1971, Mair & Tome 1972 Ghadially 1975)

In 1973 Hagelqvist and Engström demonstrated that material obtained by fine needle biopsy could be used with good results for ultrastructural studies In this preliminary paper diverse basic problems encountered in such studies were out lined and some results presented The very promising early results encouraged further study and the present publication is a direct continuation of the early paper It is now based on both experimental and clinical material The clinical material included in this book consists of tumours or other diseases of the head and neck region It is the result of collaboration between the departments of cytology and otolaryngology Important cooperation with the departments of oncology and of pathology made this study possible In a parallel experiment a number of aspirates were collected from tumours not located within the head and neck region This material is not included but provided additional information on the value of the method

The preliminary report emphasized the importance of such factors as needle diameter fixation and embedding technique etc

The investigation also indicated that the time factor inherent in electron microscopy is of great significance It is particularly important in cases of malignant tumours and certain viral and bacterial infections to find a technique which rapidly yields good results These problems were subjected to analysis Some of the questions studied can be summarized as follows

Is it possible to find a fast and reliable technique for TEM-studies of material obtained by fine needle aspiration?

The preliminary publication showed that appropriate treatment of the material can give rapid results with the specimen quality still fairly good An important question in this study is **To what extent can the TEM-method provide information on cell-structure, -function, -activity, and on interrelations between tumour cells?**

It proved possible to make rather large semi thin sections of material obtained by fine needle aspiration This possibility is further studied and will be discussed One question here is **How representative is the material, and what quantity of cells can be found in each aspirate?**

Tumour material is often easily accessible to biopsy and TEM can then be conventionally used Nevertheless in many cases it is not advisable to make a biopsy from a tumour, e g from a tumour in the salivary gland or when the aim is to follow the results of the treatment by irradiation or cytotoxic drugs An important question then arises **Will needle aspiration and TEM offer a possibility to follow the results of different kinds of treatment?**

It is established that the majority of the clinical material orginated from head and neck tumours Particular attention is paid to a study of salivary gland tumours It is of prime interest to determine **whether the present technique will add information which permits us to distinguish such tumours, and if this is the case, in what type of tumour can the method be of value.**

Our main problems are thus in abstract How should specimens be obtained and handled to give both rapid and high quality results? Can study of such material add vital information concerning tumours and tumour cells surpassing the data provided ght microscopy?

Part I MATERIAL AND METHODS

This section was produced in cooperation with Berit Engström

In this section we shall present a method for aspiration, fixation and embedding of material obtained by fine needle aspiration biopsy, which has been used in combination with conventional cytological smear techniques, often supplemented by material derived from biopsies or after operations. Particular attention was devoted to the construction of needles and to fixation and embedding. The technique was developed in animal experiments and on human material.

A ANIMAL MATERIAL

a) Qualitative tests: We began our investigation with a pilot test, by sampling material from liver and salivary glands of guinea pigs in order to find a convenient vial in which the relatively small material could be processed. In addition, certain tests on osmolarity were performed. It could immediately be shown that aspirates from liver and salivary gland gave specimens in which the well known structure of these organs could be readily recognized. We found that some of our early tests produced good specimens, which stimulated us to continue our experiments with tumour material.

b) Quantitative tests with different biopsy needles. The test aspirates were made from 5 livers and 35 kidneys from pig — about 300 liver aspirates and 1200 kidney aspirates. These organs were chosen because the liver is known by experience to yield easily aspirated material, while it is more difficult to obtain tissue fragments from the kidney, because of its toughness.

Five types of needle were tested (Fig 1D). The conventional, 0,7 mm Ø and 30 mm long injection needle (Mediplast) (A) used in everyday cytology in Uppsala. The second needle (B) is identical to the first except that the front opening was sharpened on one side on the assumption that the cutting front will increase the number of aspirated cells. The third needle (C) is a 0,7 mm Ø and 50 mm long subcutaneous needle (Mediplast). The fourth needle (D) is a 0,8 mm Ø and 40 mm long subcutaneous needle (Gilette) and the fifth needle a 0,8 mm Ø and 80 mm long injection needle (Mediplast).

Comparatively few punctures were made in liver (about 300), since it was established at an early stage that a sufficiency for embedding was obtained from this organ irrespective of the needle used for this easily punctured material. A total of 1,236 punctures were made in kidney, divided chiefly between needles A and B (709) as we were primarily interested in testing our ground needle and comparing it with the conventional A needle.

As we thought it possible, and also found by *experience*, that the needles could cause different degrees of bleeding during aspiration, all experiments which were measured and compared were made on freshly removed kidneys and livers from pigs. As far as possible each aspiration was made under identical conditions. The needle was passed back and forth seven times on a length of 10 mm, starting at the outer margin of the tissue. Then the pressure in the 20 cc syringe was equalized before the needle was retracted from the tissue. Then the tissue content of the needle was ejected into a small glass tube which had been weighed dry and empty. The aspirates were dried in a vacuum chamber with excess of drying agent present.

The dry weights of the aspirates were recorded and compared. The results from the needles B, C, D, and E were compared with that obtained by puncture with the (0,7 mm) A needle, which is used for daily routine punctures.

A histogram was plotted and mean values and standard deviations calculated (with the help of a computer). T-tests were performed.

B. HUMAN TUMOUR MATERIAL: GENERAL

The human material consists of 290 "tumours" some of which are inflammatory processes, mainly from the head and neck region. More than half of the "tumours" were localized to the salivary glands. The material was examined as follows.

a) **Conventional** smears of fine needle biopsies were stained with May-Grünewald-Giemsa for light microscopic cytological examination (Fig. 2)

b) **Aspirates for Epon-embedding** were taken (Fig. 1A), and ejected into small siliconized tubes (Fig. 1B). An Ellerman tube of the type used for Rh-determination was found to be appropriate for this purpose. At the time of ejection the needle was flushed with 2.5% glutaraldehyde. The specimen was rapidly fixed in this way and often retained its form as a small core (Fig. 1C). Washing, post-fixation, dehydration and embedding were all done in the same Ellerman-tube.

c) **Biopsy**: In cases of later surgery a thin slice from the tumour was taken at operation and divided into small pieces of about 1 mm³. These specimens were fixed in 2.5% glutaraldehyde and embedded in Epon 812 (Luft 1961) in the same way as the fine needle aspirations.

C. HUMAN MATERIAL. TESTS FOR REPRESENTATIVITY

In an effort to analyse whether the aspirated material could be regarded as representative for the tumour examined a comparison was made with material obtained from "surgery"-biopsies from the same case. For this investigation we chose tumours which were diagnosed from cytological smears as adenoid cystic cancer (ACC) or highly suspect ACC and studied them as follows.













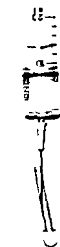




Fig 1
A Fine needle aspiration with Frazén syringe
B Specimen being ejected into Ellerman tube
C Aspirate in fixation fluid (glutaraldehyde)
D Different kinds of needle-opening

a) Qualitative tests

One fine needle biopsy was taken from each of three tumours, and these aspirates were fixed and Epon embedded. Each was sectioned for TEM at five different levels — 15 different areas in all. Later, at surgery, a thin slice was cut from each of the three tumours. These slices were divided into small pieces. Five of these "surgery"-pieces were chosen at random from each of the tumours. Sections for TEM were cut from each of these small pieces — 15 areas in all. The areas sectioned were trimmed down to equal that of an aspirate specimen in area. About 12 micrographs at a magnification of 2000 X were required to cover every single TEM-section. The micrographs from aspirated and surgery material were mixed. Three experienced investigators tried independently to separate prints from aspirated material from those of surgery material.

b) Quantitative tests

In a effort to make a semiquantitative study of the cell material obtained by aspiration relative to the material derived from biopsy, seven cases of cytologically suspect or certain ACC were investigated. In sections chosen at random from both aspirate and biopsy 200 neighbouring cells were studied from each case. The cells were separated according to the criteria given below and histograms made. As will be further described in Part III, five cell groups were differentiated, mainly according to descriptions by Chisholm *et al.* (1975) and Hoshino and Yamamoto (1970). The following symbols were chosen: L-cells (light cells), L_i cells (intermediate cells), D cells (dark cells), D_i cells (intermediate cells) and Sq cells (squamous epithelium cells).

D. FIXATION, EMBEDDING AND SECTIONING. GENERAL

a) Standard technique

In most cases we used 2.5% glutaraldehyde in 0.12 M phosphate buffer with pH 7.3 as fixative. In some cases we used cacodylate buffer but as its toxicity has been widely discussed, we preferred the phosphate buffer (Glauert 1975).

The fixation time varied from 1 to 4 hours (in room temperature). In a few cases the specimens were left in the glutaraldehyde for more than 48 hours, which did not seem to cause any major damage. After fixation the specimens were washed in the buffer or in isotonic saline, three changes during 1 hour. Postfixation was invariably carried out in 1.5% veronal acetate buffered OsO_4, pH 7.3 (Palade 1952, Zetterqvist 1956, Glauert 1975) for 1 hour. The specimens were then washed three or four times during 30 minutes in isotonic saline. They were then dehydrated in ethanol of increasing concentrations (45—60 min) and finally brought into propylene oxide (2 × 15 min). In some cases, where time factors influenced the technicians work, we left the specimens overnight in 70% ethanol. The specimens were infiltrated in 1:1 mixture of propylene oxide and Epon 812 for at least 50 min. Then the lids were removed to allow the propylene oxide to evaporate before final embedding. Where a simultaneous embedding of several specimens was planned, the specimens first





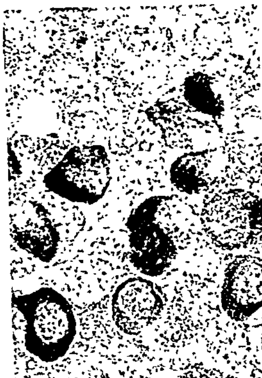




Fig 2 Samples of some typical cytological smears.
A from pleomorphic adenoma of the parotid
B from poorly differentiated cancer from the parotid. Observe the pseudoinclusion in a nucleus.
C Metastasis from malignant melanoma Masson stain.
D Cancer in the parotid gland, presumably of endocrine origin (compare Fig 25)

collected were left in propylene oxide — Epon mixture for up to a few days The final embedding was made in Epon 812 with polymerization for eight hours at 40°C, followed by 60°C for two days

b) Fixation and embedding for a "rapid" diagnosis

When rapid processing was desired we used a technique for fixation and embedding modified after Johannesen (1973), Hayat & Guiaquinta (1970), Bencosme & Tsutsumi (1970) The specimens were fixed in 2 5% glutaraldehyde in 0 12 M phosphate buffer for 30 min at room temperature,followed by careful washing in isotonic saline Postfixation was done in 1 5% OsO_4 in veronal acetate buffer for 45 min The specimens were dehydrated in increasing concentrations of ethanol for 40 min, followed by two changes of propylene oxide (2×5 min) and one change of propylene oxide and Epon in 1 1 concentration (20 min) The specimens were embedded in Epon 812 and polymerized at 70°C for 45 min, then in 105°C for 75 min After polymerization the glass tube around the Epon often breaks spontaneously or will break if rapidly immersed in ice water after which the glass is easily removed When it remained intact, we cracked the tube in a vice with flat faces The specimen can, with these times, be embedded within 5—6 hours A somewhat slower method suitable for less urgent cases was also used This is not as time-consuming and the preparation can stand polymerizing overnight, and be ready to section the next morning

Table I illustrates the three methods used

Processing	"Rapid" 5—6 h	"Semi rapid" 18—24 h	Standard 2—3 days
2 5% glutaraldehyde	30 min	1 h	1—4 h (48 h)
Buffer or isotonic saline	3×10 min	1 h	1—2 h
1 5% OsO_4	*30—45 min*	*1 h*	*30 min—1 h*
Buffer or isotonic saline	3×10 min	1 h	30 min—1 h
Dehydration			
Ethanol 50%	2×5 min	2×5 min	5 min + 10 min
70%	2×5 min	2×5 min	5 min + 10 min
95%	2×5 min	2×5 min	5 min + 10 min
100%	2×5 min	2×5 min	5 min + 10 min
Propylene oxide	2×5 min	3 min + 15 min	2×15 min
Propylene oxide Epon 812 1 1	20 min	30 min	50 min a few days
Polymerization	70°C 45 min followed by 105°C 75 min	70°C 45 min followed by 95°C 12 h or overnight	40°C 8 h followed by 60°C 2 days

All stages are carried out at room temperature

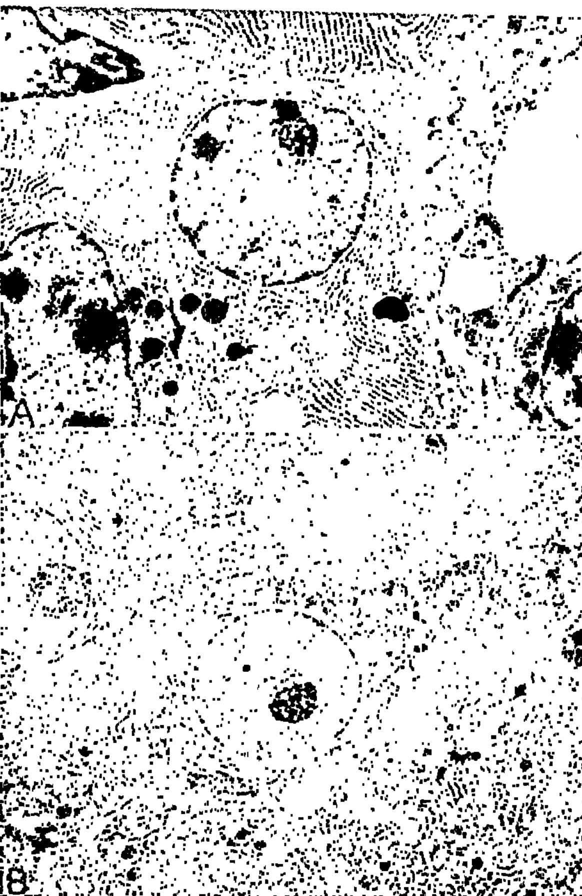
Osmolarity investigations

The primary fixation consisted almost exclusively of of 2 5% glutaraldehyde in phosphate buffer, followed by secondary fixation with OsO_4 in veronal acetate buffer At the beginning of the investigation studies were made with regard to suitable osmolarity of the phosphate buffer, these were performed on guinea pig salivary glands (Fig 3) Later in the course of the investigation two further series of tests were carried out on the same tissue, in which the molarity of the phosphate buffer varied between 0 06 and 0 19 M, with gradual changes of 0 01 M On this material from normal salivary gland tissue the cristae and membrane systems of the mitochondria were, as a rule, best preserved at values between 0 12 and 0 14 M, corresponding to an osmolarity of 280—310 mOsm It has long been known that the osmotic pressure differs in different cells and likewise in different tumours (Palade 1956) In our tumour material we normally used a phosphate buffer which was 0 12 M, corresponding to an osmolarity of 280 mOsm Naturally it was impossible to determine whether poor quality in membranes and mitochondria in a specific case depended on wrong osmolarity, since so many other factors in the embedding of the material influence the final quality

By application of the **rapid embedding technique** the specimens could be made ready for electron microscopy within five hours of aspiration The duration of fixation in glutaraldehyde could be reduced to 15 min, and the period in OsO_4 to 30 min, without major impairment of quality The period of dehydration, however, must be maintained We found no major structural differences in rapidly processed specimens when compared with material subjected to routine treatment (Fig 4) We used the rapid process in more than 20 cases The speedy processing does not seem to have any serious disadvantages if the specimens are small and the change from propylene oxide/Epon mixture to pure Epon carefully carried out to ensure that no propylene oxide remains Thus the technique can be used for swift diagnosis when the time factor is crucial In cases where the timing is less important, however, it is necessary to develop techniques where the methodology is controlled by the dictates of quality

Semi thin sections

In the preparation of sections for TEM it is usual to make semi thin sections first, in order to locate special regions for ultra thin sectioning This is necessary when representative regions are desired or the material is small and perhaps mixed with blood The material obtained by fine needle biopsy may, as stated vary considerably in amount, but specimens frequently contain much more material than was expected In such cases when the material is sufficient, a study of semi thin sections (1—3μ) may be of value It is common to find large numbers of cells well fixed and interrelated with neighbouring cells The cells are often arranged in large clusters sometimes retaining the cyto architecture of the tumour An example is illustrated in Figs 5 and 6 A As a single semi thin section may contain several hundreds to thousands of cells it offers an opportunity to observe many details such as form and


A



Fg 4 M crograph from a case of mal gnant lympho-ep thel al les on where desmosomes (open arrows) are found betw een the cells
Th s spec men is handled accord ng to the rap d embedd ng techn que M crographs of th s qual ty can be made w th n 5—6 hours after aspirat on (6500×)

Fg 3 Two micrographs from our p lot stud es A Normal salivary gland from gu nea p g (6500×) B L ver puncture from a pat ent w th symptoms of reject on of his transplanted k dney (4500×) These two m crographs are to demonstrate that the general texture is reta ned in the aspirate

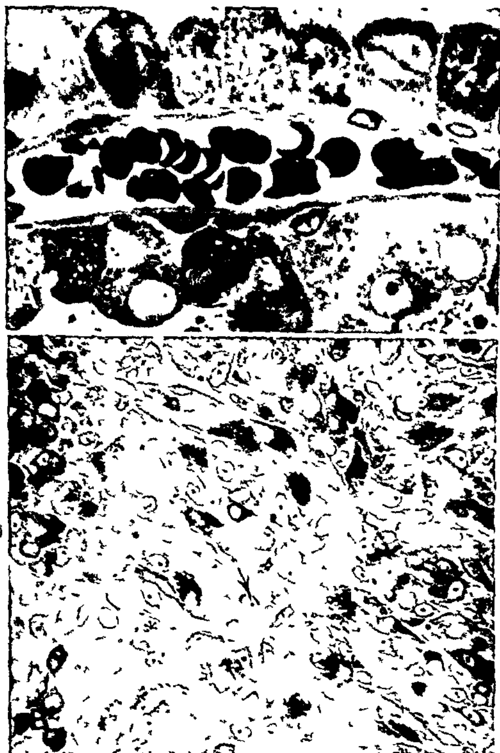


Fig. 5. Epon embedded tumour material, semi-thin sections. A. Adeno-papillary cancer of the parotid gland (1550×). B. Unclassified cancer of the parotid gland ([illegible]00×). Phase-contrast microscopy. These micrographs demonstrate that it is possible to get good information on a tumour and its cells. Many details of the cell cytoplasm, nuclei (arrow) and nucleoli (*) can be recognized. Certain information concerning the cellular arrangement is also obtained.

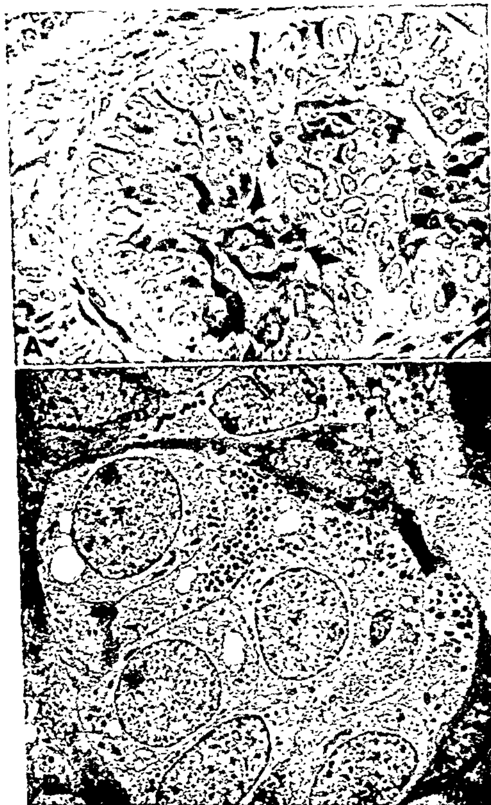


Fig. 6 Micrographs from a case of sialosis in the parotid gland. A is a semi-thin section and B a TEM-micrograph. There is good agreement between light and electron microscopy (A 710×; B 4100×).

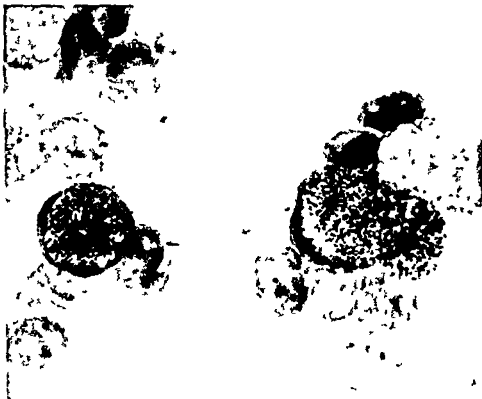


Fig 7 Autoradiography of tritiated thym d ne in a case of malignant lymphoma The uptake of isotope is visual zed by the dark gra ns in the cells Aspirate and l ght m croscopy

size of the cells and their nuclei, cytoplasmic granules or inclusions, relation to ves sels or microcysts When we began our studies we used these sections for TEM localization only

We found, however, that they can be of interest for the evaluation of the tumour as well A typical example is seen in Fig 31 B showing a semi thin section from an adenoid cystic cancer which had been irradiated Material from the operation showed no tumour cells present, while our semi thin section clearly reveals the pre sence of tumour cells and the characteristic pseudocysts with their basement membrane like wall

During our studies of the aspirates, and also when studying materials from opera tions we had access to various methods not described here These include autoradio graphy with tritiated thymidine and scanning electron microscopy (SEM) We have not included this part of our material, but Fig 13 B illustrates a SEM micrograph It should be mentioned that for special purposes, such as mycosis or the study of surface structures, the SEM technique may be an important adjunct to other met hods here described Autoradiography with tritiated thymidine was used in the study of the results of irradiation treatment but the evaluation of this material involved elements of uncertainty and our ivestigations must therefore be continued Nevert heless we were able to demonstrate that aspirated material can be used for at least light microscopic studies of this type This technique will aslo be studied further

A. CELL STRUCTURE GENERAL.

Clinical diagnosis of tumours was for many years dependent mainly on light micro scopic observations, but during the last decade more and more information on tumours and tumour cells has been derived from electron microscopy Transmission electron microscopy was the chief technique used in these studies The combined use of light and electron microscopy has enriched our knowledge In the present study we confirmed Ghadially's finding concerning tumour cells "It is amazing how much more one can discern with the light microscope once their ultrastructural fea tures are recognized" The cytological study of smears from tumours is to a great extent based upon the form and structure of cell nucleus and cytoplasm, and the interrelationship of tumour and host cells The same factors are of great interest also in TEM studies, but the possibility of discerning the detailed inner structure of the cell nucleus and cytoplasmic organization adds a new dimension

Diverse important factors appear in the literature with regard to TEM studies of material from surgery specimens, but also in the present investigation, factors which are of diagnostic interest which may become more so as our knowledge of their importance increases

A striking feature is the detailed information on cell borders (Fig 8) and cellular relationships which TEM can offer TEM provides an excellent opportunity of observing cell junctions and interdigitations (Fig 9), while cytoplasmic organelles such as microvilli, inclusions etc can be radily discerned on free cell surfaces

Many authors have reported a reduced number of junctions between some types of tumour cells and wider intercellular spaces in some tumours have also been observed On the other hand, a positive finding of many desmosomes in a tumour of uncertain etiology may be of importance in the differential diagnosis between a car cinoma and a sarcoma In Fig 4 presence of desmosomes in a case of poorly diffe rentiated salivary gland tumour excluded a malignant lymphoma, and contributed to the final diagnosis of malignant lymphoepithelial lesion We encountered a few cases with this diagnosis, and they are now under further study TEM examination of the cell surface of tumours may show special cytoplasmic features, such as cilia and microvilli, as indications of differentiated surface activity (Fig 29 B) A loss of such specialized cell function may be a sign of malignancy Similarly an increased incidence of atypical microvilli, seen e g in "hairy leukemias" (Bessis 1977), may be of significance for the diagnosis



Fig. 8 Myeloma cell, from the bone marrow, fine-needle aspirate. This micrograph shows how TEM permits a study of the structure of cell nucleus and cytoplasm. Mitochondria (*M*), endoplasmic reticulum (*Er*) and cytoplasmic granules (*G*) as well as cytoplasmic protrusions (*P*) from the cell surface are evident (14400×)

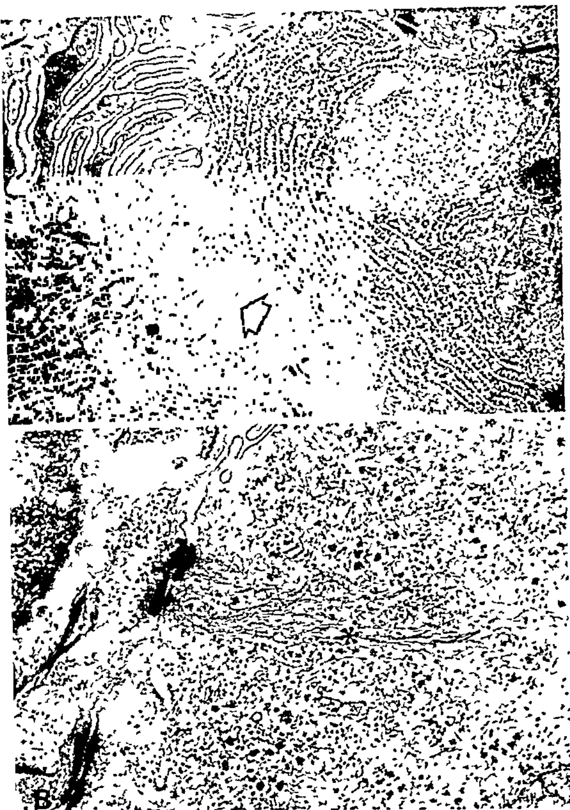


Fig 9

A TEM-micrograph showing interdigitation (arrow) between two salivary gland cells (31000×)

B Desmosomes with long tonofibrils(*) seen in a case of pleomorphic adenoma (45500×)

B. SPECIFIC CELL STRUCTURES AND VIRUSES

The cell surface is not the only subject of interest, since the form and structure of the cell nucleus emerges more distinctly in TEM than in light microscopy It is asto nishing how often light microscopy implies that the nucleus of a tumour cell is a regular sphere, whereas TEM proves that many are irregular and indented, and per haps contain pseudo inclusions Three nuclei and various pseudo inclusions are illustrated in Fig 10 The full significance of the structure of the complicated infol dings in Fig 10 A or the protrusion in 10 B cannot yet be understood It is assumed to correspond to wider area of contact between the nucleus and the surrounding cytoplasm of importance for nucleocytoplasmic exchange, or is perhaps an indica tion of enhanced metabolic activity

Pseudo inclusions in the nuclei are comparatively common in malignant tumour cells, and were observed in myelomas (Fig 10 B and C), in malignant melanomas, and in papillary carcinomas of the thyroid These pseudo inclusions can be clearly discerned even in ordinary cytological smears studied under the light microscope, (Fig 2 B) but only when using TEM is it possible to distinguish the nature of the structures contained within them A high frequency of pseudo inclusions in naevi and malignant melanomas was descibed by Sobel et al (1969), who found them in 8 of 10 malignant melanomas, and 27 of 34 naevi

The chromatin of the nucleus in our specimens varied widely in different kinds of tumour and also in different parts of the same tumour Presumably increased know ledge of chromatin and nucleolus structure will improve our possibility of evalua ting the potential growth of tumour cells

In the studies one of the prominent features revealed by TEM was the form of the nucleolus in individual tumours Enormous nucleoli could be seen in a case of maxillary cancer (Fig 11) In other cases we observed a rich variety of nucleolar structures in different types of tumour, and even in tumours where the clinical diag nosis was the same Different types of nucleoli are illustrated in Fig 12 Figure 12 A shows an enlarged, centrally situated nucleolus, in contrast to that in Fig 12 C, which displays "nucleolar margination" in a tumour cell from a malignant lympho epithelial lesion in the parotid gland The latter form was regarded by Ghadially (1958, 1971, 1975) *et al* as a sign of rapid cell growth and increased protein synthe sis Nucleoli of the type illustrated in Fig 12 B were observed by Luse (1961) in cer tain brain tumours

A less common intranuclear inclusion is shown in Fig 13 This "concentric lami nated inclusion" was found in an aspirate from a submaxillary gland infected by actinomyces Israeli The importance of such inclusions, seen by Tandler and Den ning (1969) in mucus producing cells in human salivary glands, and by Erlandsson and Tandler (1972) in adenocarcinomas of the parotid gland is not known

We observed another spiral like inclusion in a case of malignant lympho epitheli al lesion Here too the significance is obscure Similar inclusions were seen by Ze lickson and Lynch (1961) in a case of kerato acanthoma, and classified as caused by a virus Similar nuclear bodies were described by Bouteille et al (1967) as evidence of cellular hyperactivity of multiple origin



Fig 10 Three micrographs demonstrat ng the structure of the nucleus w h TEM on fine needle asp ra tes A is a very rregular nucleus n a case of poorly d fferentiated sali ary gland cancer (12900×)
B s a pseudo inclus on (arrow) nto the nucleus (*Nu*) of a myeloma cell (2400×) and C is the irregular nuclear envelope (arrow) in myeloma cell from the same case as B (2400×)

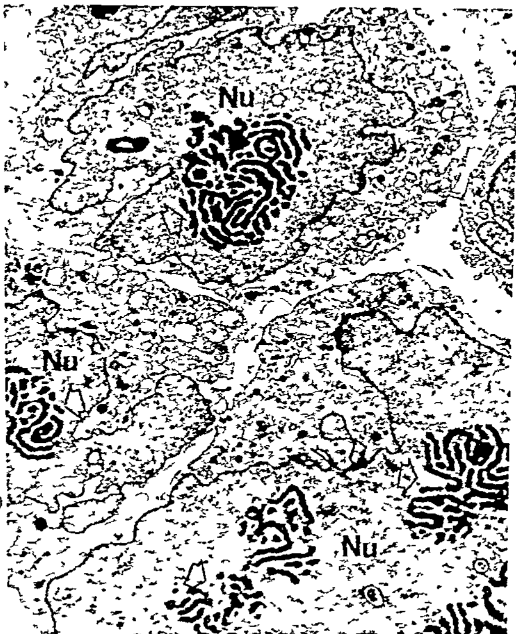
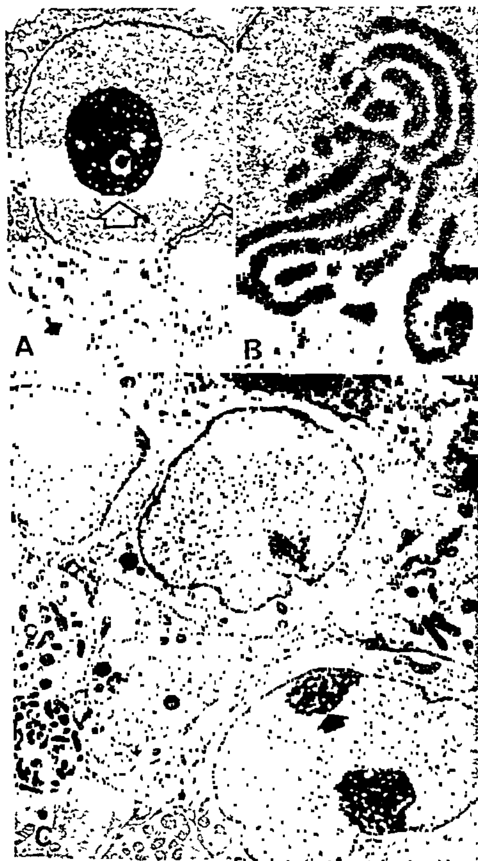


Fig 11 TEM on an aspirate of a rapidly developing maxillary cancer with large nucleus (*Nu*) and enormous nucleolar structures (arrow) This case showed many mitotic divisions and the micrograph indicates high mitotic activity (7100×)

Fig 12 Three micrographs present different forms of nucleoli A is from a case of metastatic sarcoma (7000×), B from a maxillary cancer (24000×) and C from a malignant lymphoepithelial lesion (10400×) Arrows in A and C point at the nucleoli


A
B
C

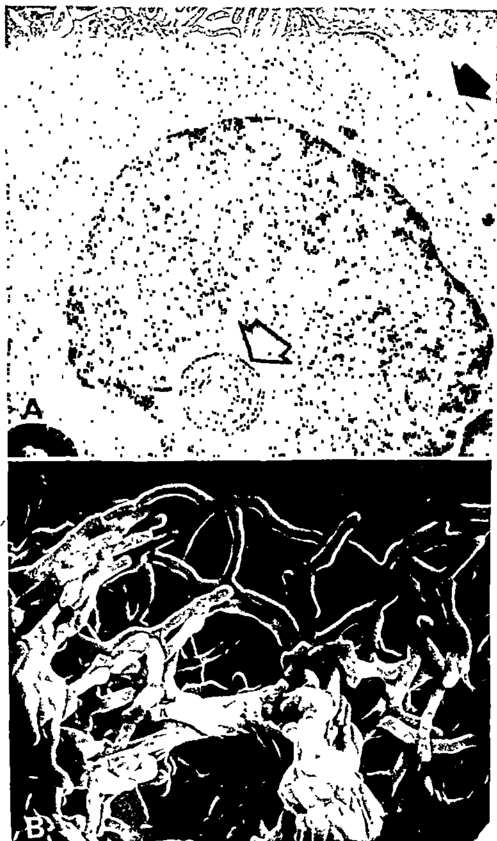


Fig 13 A TEM micrograph and B Scanning electron micrograph from a case of actinomycosis (*Actinomyces Israeli*) In A the open arrow indicates an intranuclear concentric inclusion and closed arrow cell junctions, (18400×) in B the mycelium can be seen (8500×)

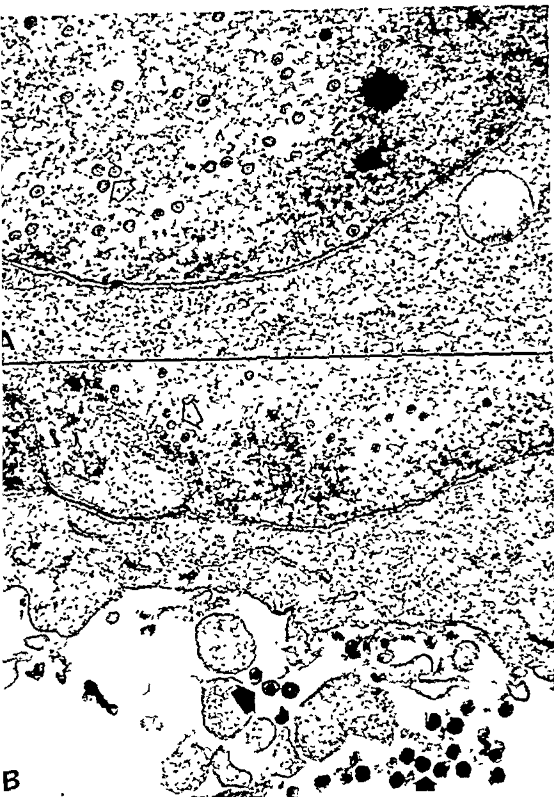


Fig 14 Two TEM-micrographs showing viruses in a case of herpes encephalitis Fine needle aspirate and TEM was carried out in this case *which was supposed to* be a brain tumour With the rapid method we could within 6 hours demonstrate virus in TEM (In A there are viruses in the nucleus and in B are seen viruses both in the nucleus and extracellulary (A 36 400×, B 28 000×)



Fig. 15. Vesiculated endoplasmic reticulum (*Er*) surrounding a Russel body-like structure in a case of myeloma. It is probable that the reticulum is distended by newly formed pathological protein. (24000 ×).

Viruses represent a special nuclear or cytoplasmic inclusion which can be directly recognized by TEM. An example from our material is illustrated in Fig. 14, where fine needle aspiration performed at operation of a case of suspected brain tumour, followed by TEM, showed viruses. In this case the rapid embedding method was used, and TEM examination took place within six hours after aspiration. The virus was of the *herpes simplex* type. The micrograph shows that inside the nucleus the central core of the virus is delimited by a so-called capsid. To the viruses which have left the nucleus one more layer has been added by the nuclear membrane.

The cytoplasm of cells in the aspirates may vary in quality of fixation but this is generally good, so that cytoplasmic organelles can be studied in detail. The rough endoplasmic reticulum (Er) is clearly defined in Figs. 8 and 15. In this case the plasma cell from a myeloma shows many other details of cytoplasmic differentiation.

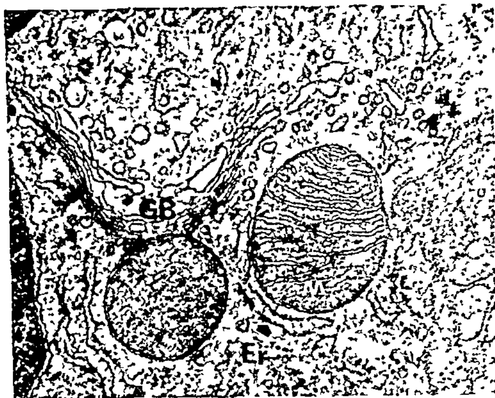


Fig 16 Mitochondria (*M*) rough endoplasmic reticulum (*Er*) and a Golgi complex (*GB*) from a case of mucoid adeno-papillary cancer in a salivary gland (46000 ×)

The presence of a well developed endoplasmic reticulum is regarded as evidence of advanced RNA synthesis A rich rough endoplasmic reticulum is a normal finding in many secretory cells, as in salivary glands (Fig 3 A and 9 A) The reiculum may vary considerably in form and extent in tumour cells, which is presumably directly related to protein synthesis

Another tumour with pronounced secretory capacity and consequently a well developed endoplasmic reticulum is seen in Fig 16, which shows a tumour from a mucous adenopapillary salivary gland cancer There is also a well developed Golgi complex and secretory vacuoles signifying high secretory production

Another arrangement is demonstrated in Fig 17 B where the endoplasmic reticulum encloses the mitochondria, probably to allow of a more rapid exchange of energy A rich endoplasmic reticulum is shown in Fig 17 C, which is a TEM microphotograph derived from an aspirate from a lymphatic gland metastasis in the neck from an endometroid cancer Light microscopy of the cytological smear revealed an abundance of "lymphoglandular bodies" (Söderström 1966)

Yet another type of "lamellar bodies" almost resembling smooth endoplasmic reticulum derived from a liver tumour is illustrated in Fig 17 A



Fig 17 Three different forms of endoplasmic reticulum A, An aspirate from a hepatoma, B, A mucous adenopapillary carcinoma in the parotid gland and C, a lymph node metastasis of an endometroid can

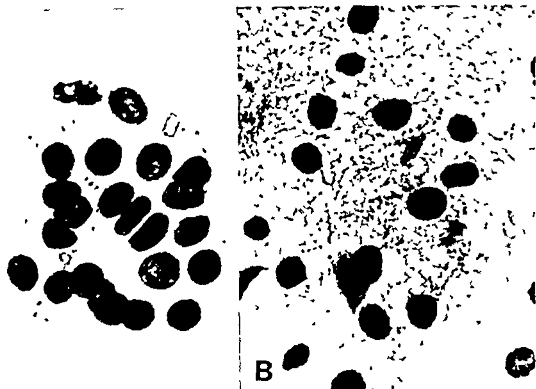


Fig 18 Light microscopic pictures from smears of (A) oncocytoma and (B) acinic cell cancer Compare the TEM micrographs *Figures 19 and 20* (A 925 ×, B 925 ×)

As many of these structures have a real bearing on the function of the cells, and presumably also on their malignancy, a thorough knowledge and further information are of great interest Indeed it may be that many of these features have their main significance as evidence of different grades of cellular activity

Moreover the cytoplasm may contain structures of a specific nature which is of importance for the diagnosis of a tumour

In cytological smears for light microscopic examination it may sometimes be very difficult in certain salivary gland tumours to determine whether the granulation of the cytoplasm in tumour cells is caused by e g serous granules, mitochondria or lysosomes, a judgement which alone decides whether it is e g an acinic cell cancer (Erlandsson & Tandler 1972), an oncocytoma (Tandler et al 1970) or a Warthin tumour (Tandler & Shipkey 1964) or a granular cell myoblastoma (Sobel et al 1972) The cells which seem alike in light microscopy (Fig 18) prove to be completely different in TEM In acinic cell cancer (Fig 19) the cytoplasm displays large numbers of, in part, atypical densely clustered secretory granula of varying size The oncocytoma cells show (Fig 20) an accumulation of pathological mitochondria, and the granular cell myoblastoma a multitude of, inter alia, lysosomes

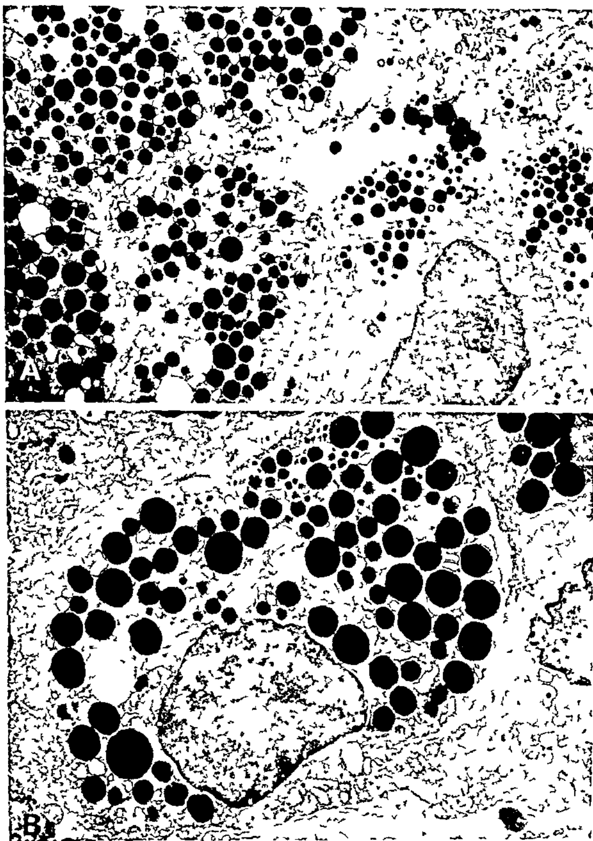


Fig 19 A and B show secretory granules filling the cells in a case of acin c cell cancer Many of the cells in this form of salivary gland tumour have a close resemblance to normal secretory cells This is not at all the case in oncocytomas and adeno lymphomas Compare *Fig 20* (A 6 500 × B 9 000 ×)

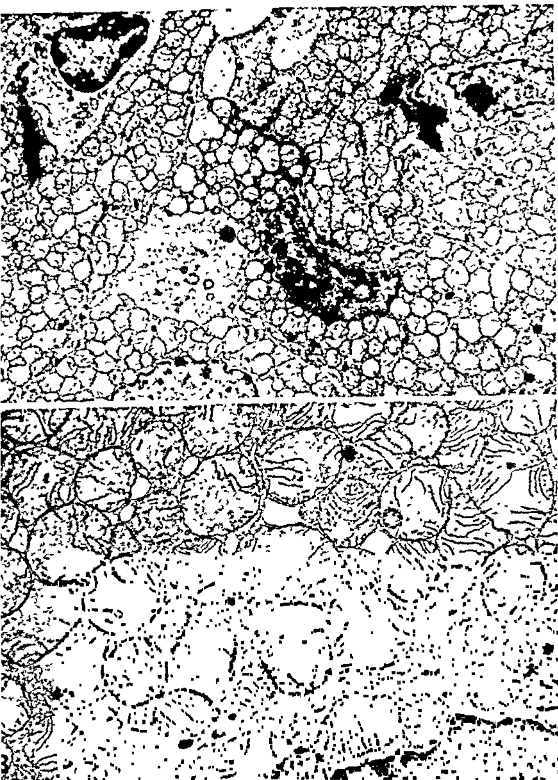


Fig 20 A and B Cells filled with pathological mitochondria in a case of oncocytoma It is interesting to compare this case with the one shown in *Fig 19* where the cells are packed with granules
These two tumours are often difficult to differentiate on smears for light microscopy (A 5500×, B 19000×)

Mitochondria in tumours studied by TEM can be observed in far greater detail In some tumours the mitochondria may be almost normal in appearance, with regular borders and cristae Other highly atypical and sometimes characteristic mitochondria were observed in our TEM aspirates Various forms of atypical mitochondria are illustrated in Fig 21 Figs 21 B, C and D are particular interest since their appearance impelled us to suspect a renal cancer which was thus clinically diagnosed These mitochondria reveal bizarre formations with their cristae often concentrically layered Elongated, club like, and dough nut shaped mitochondria were also seen in this case

These findings in renal cancer are described by, inter alios, Seljelid and Ericsson (1956) and by Keyhani (1969), but are nevertheless not specific for this type of cancer The case will be further discussed later

In several cases the appearance of the mitochondria in tumours may not be characteristic, but merely show a deformation due to a degenerative process Such mitochondria can be seen in Fig 21 A A similar type of structural modification has been observed in different kinds of tumour These changes often appear in the form of myelin figures, which by some authors are regarded as a fixation artefact (Ericsson and Biberfeld 1967)

Some of the cytoplasmic inclusions may be highly characteristic and some can be observed by both light and electron microscopy, such as the Charcot Leyden crystal in Fig 22, or the eosinophilic granule in the same figure The aspirate was taken from a skeletal destruction in the temporal region of a woman where both X ray and cytological smears made the diagnosis of eosinophilic granuloma strongly suspect

As a rule the pathologist or cytologist can reach his diagnosis with the help of routinely prepared slides for light microscopy as here Difficulties may arise, however, with regard to poorly differentiated tumours With the help of TEM it is at times possible to identify persisting intracytoplasmic structures as characteristic of the tissue of origin The amelanotic malignant melanoma often difficult to diagnose is a tumour of this type

TEM can disclose immature stages of the melanosomes in the form of premelanosomes (Melanosome stage II according to Fitzpatrick et al 1971) which allows of confirmation of the diagnosis Figures 23 A and B illustrate melanosomes of differing degrees of maturity in the form of, inter alia, premelanosomes, with their typical zig zag pattern and more mature granules containing amorphous dark melanin (Melanosome stage IV) (Fig 23 C) Other structures which are difficult to discern in light microscopy but have a characteristic picture in TEM are amyloid Fig 24 depicts aspirate from a lymph gland metastasis in the neck from a medullary thyroid cancer where the diagnosis could not be reached solely on the cytological smears TEM allows clear perception of densely packed amyloid fibrils In Fig 24 A and B it is also possible to discern granules which are interpreted as being of endocrine nature The calcitonine titer proved to be high in this patient The diagnosis medullary thyroid cancer may therefore be regarded as certain

The question of whether the salivary glands also exert endocrine activity was asked long ago, inter alios by Feyrter in 1961 One of our salivary gland tumours, which was primarily diagnosed from smears as an atypical pleomorphic adenoma (Fig 2 D) and on histopathological determination classified as cancer in a pleomorphic adenoma, showed in TEM plentiful accumulations of relatively small granula (Fig 25) They did not resemble ordinary secretory granules in appearance but provoked suspicion of endocrine activity Silver staining according to Grimelius (1968) was positive, so that the TEM finding could be confirmed The case is further studied and will published separately

A number of other, non specific, cytoplasmic inclusions are illustrated in Fig 26 Fig 26 A shows a secondary lysosome/phagolysome, and primary lysosomes and presumably lipid droplets

Ordinary cytoplasmic inclusions are glycogen particles which, however in TEM may often be difficult to distinguish from polyribosomes, or even artefacts such as lead deposits Fig 26 B shows an abundance of glycogen in rosette formations, from a liver puncture Fig 21 C and D also reveal that the glycogen may be located inside a pathological mitochondrion in cases of suspected hypernephroma metastasis Seljelid and Ericsson (1965) demonstrated this pronounced accumulation of glycogen in the mitochondria in renal cancer Nevertheless already in 1964 Tandler and Shipkey showed that the same glycogen incorporation can be seen in the mitochondria in Warthin tumours Similar mitochondrial changes can also be perceived in a large number of myopathies

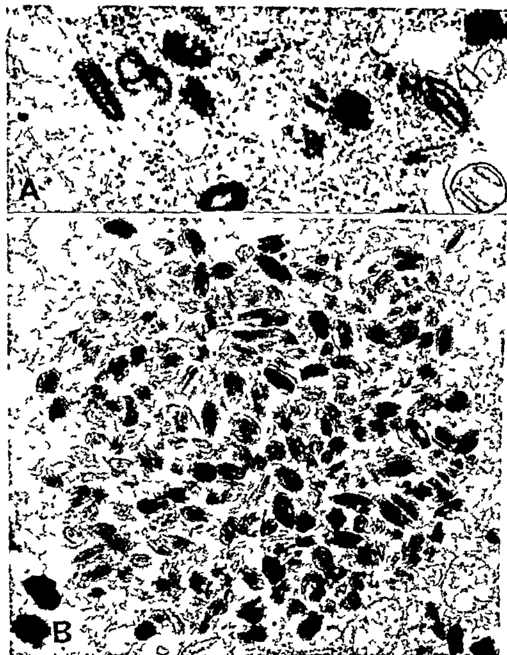


A
C
D



Fig 22 A shows a TEM micrograph from a case of eosinophilic granuloma in the temporal bone with an eosinophilic granule (*Eos*) and a Charcot Leyden crystal (37 500×) B shows mucous granules in the submaxillary gland of a patient with sialo adenitis (17 500×)

Fig 21 Atypical or pathological mitochondria from A a case of malignant lympho-epithelial lesion and B C and D a case of multiple salivary gland tumour This case was histopathologically classified as "acinic cell cancer" *Against the background of the form and appearance of the mitochondria as found by TEM on an aspiration from the parotid gland a renal tumour was suspected and X ray of the kidneys showed bilateral renal tumours This was also verified by cytologic aspirate*
(A – 45 500×, B = 25 000×, C = 30 000×, D = 14 000×)



F g 23 TEM m crographs from a lymph node metastasis of mal gnant melanoma
A Premelanosomes (arrow) w th typ cal z g zag arrangement (80000 ×)
B Agglomerat on of premelanosomes and melanosomes (32500 ×)

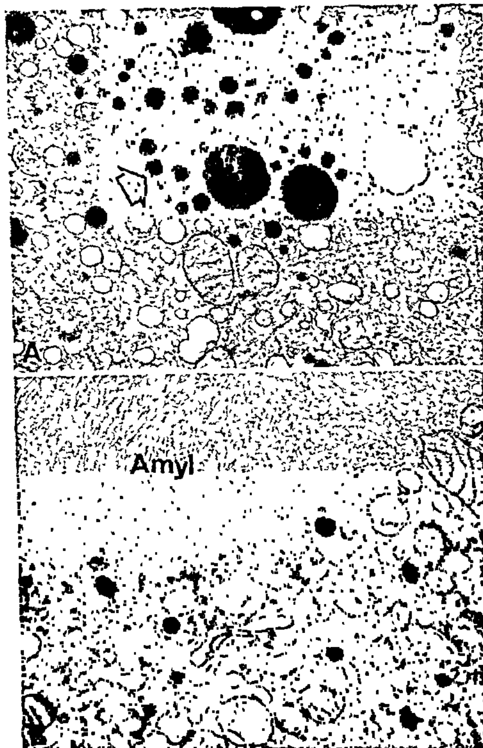
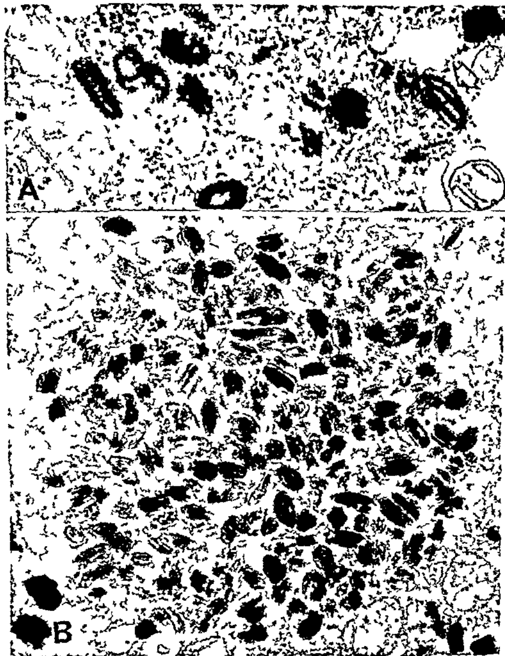


Fig 24 These two micrographs are from a case of medullary thyroid carcinoma In A cytoplasmic inclusions (arrow) have been supposed to be evidence of endocrine production, (32 500 ×) In B typical amyloid (*Amyl*) can be seen close to or in a cell with much deformed cytoplasm, (29 000 ×)



F g 23 TEM m crographs from a lymph node metastasis of mal gnant melanoma
A Premelanosomes (arrow) w th typ cal z g zag arrangement (80000×)
B Agglomerat on of premelanosomes and melanosomes (32500×)

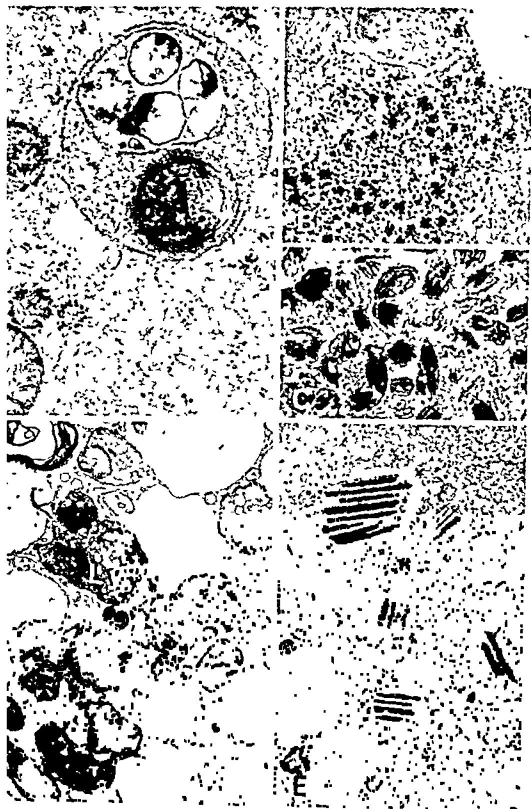




Fig 27 Two micrographs from a case of pleomorphic adenoma In A, the cyto-architecture of the tumour is seen in a specimen taken at the operation In B, the TEM-micrograph from an aspirate in the same case gives a picture showing good agreement (A 200×, B 1800×)

INTRODUCTION

Tumours of the salivary glands are sometimes difficult to diagnose because they display highly variable histological structures Their classification therefore remained obscure for many years The new WHO classification was established in 1972

Adenoid cystic cancer (ACC) has long been controversial and difficult to classify First described by Billroth in 1859 it was then called cylindroma, a name based on a structural description This name was later used also for more benign types of tumour, such as hidradenoma and various forms of basal cell cancer In 1930 Spies introduced the term ACC, which was not generally accepted until 1954, in connection with Foote and Frazell's classification of salivary gland tumours Thackray and Lucas (1974) gave a detailed description of the many variants of ACC, from the most typical stroma rich types with cystic or cribriform structures to the more solid manifestations with indeterminate structures (Fig 28)

The pre-operative diagnosis of salivary gland tumours is important for the planning of an operation This applies not least to ACC, which was regarded for many years as a comparatively benign tumour, by reason of the usually good five year survival rate A carefully analysis of ACC shows, however, that it is possible to distinguish varying degrees of malignancy Thus investigations by Eneroth and Hjertman 1966, Blanck et al (1967), Eneroth (1970, 1976) and Blanck (1976) demonstrated that it can be highly malignant, especially when appearing predominantly solid in structure Knowledge of the diagnosis of the tumour is of special interest for the planning of surgery, considering furthermore that according to certain authors (Blanck et al 1967) ACC is radiation sensitive, and that radiotherapy should be undertaken pre-operatively

For pre operative diagnosis various oncologists have resorted to coarse needle biopsies ad modum Silvermann (1938), and surgery biopsies But these biopsy techniques proved to involve a risk of local metastasis by implantation, which may complicate subsequent surgery and increase the possibility of relapse Biopsy by means of fine needle aspiration, however, proved to involve very little risk in this respect (Engzell et al 1971) Using a comprehensive material these authors could demonstrate that the risk of metastasis after fine needle biopsy was negligible Moreover the patients examined included 157 cases of pleomorphic adenoma (Fig 27), followed up for 10 years after puncture In no case was it possible to prove that the puncture gave rise to dissemination of tumour cells Three relapses which were observed were thought to result from non radical primary surgery Fine needle biopsy for pre operative clinical diagnosis of tumours has, as already pointed out, been in use in Scandinavia for many years Studies on the salivary gland include Berge and Söderström (1963), Eneroth and Zajiceck (1965, 1966), Mavec et al (1964), Eneroth et al (1967 and 1971), and Zajiceck (1974)



Fig 28 Light microscop (A) and electron m roscop c picture (B) from a case of adeno d cystic cancer (ACC) In A the typical cr b form appearan e of the tumour is evident and in B the structure of a pseu docysts (*PC*) can be seen In de the p eudocysts there are several basement membrane l ke layers (ar row) and also some cell remnan (A 200 × B 6 300 ×)

Only a few papers have been published on cytological diagnosis of ACC One of the more extensive was carried out by Eneroth and Zajicek (1969), who showed that in 25 of 45 cases of ACC diagnosis could be made primarily on the incidence of well defined "spherical bodies" or "mucoid globules" which in May Grünewald Giemsa (MGG) staining took on a dark red colour These structures were chiefly seen when the tumour was cribriform in its mode of growth Furthermore, they considered that in such cases the diagnosis was easy to make by cytology When the tumour is of the more solid type it is more difficult to reach the correct diagnosis

According to several authors fine needle aspiration biopsy in the hands of an experienced cytologist gives just as representative a material as coarse needle biopsy and surgery biopsies without their disadvantages (Zajicek 1974 and Eneroth 1976) Moreover surgery biopsy from salivary gland tumours is considered by many to be a contra indication, because of the risk of implantation

Nevertheless we must admit that it is sometimes difficult or impossible to give a diagnosis even when aspirated material is sufficient and frozen sections are done as well It might even be difficult to determine whether the tumour is benign or malignant Therefore it is of great interest to increase our knowledge of these tumours

The introduction of electron microscopy has given further essential information on the inner structure of tumour cells, and certain organelles have been found to be specific for some tumours (Ghadially 1975) These TEM investigations were carried out mainly on surgery material

On basis of the experience gained with TEM in tumour biopsies, Hagelqvist and Engström (1973) made an electron microscopic study of material obtained by fine needle aspiration biopsy, and demonstrated that TEM can be performed with qualitatively good results in salivary gland tumours These preliminary studies showed that the method would be a valuable supplement to the pre operative diagnostics when the conventional cytological diagnosis was doubtful or insufficient Certain salivary gland tumours may have a similar cytological appearance, whereas TEM examination may reveal different intracellular structures of importance for differential diagnosis This is the case e g in decision on the diagnosis acinic cell cancer contra oncocytoma, which may closely resemble each other cytologically Ultrastructurally it is easily shown that large numbers of atypical mitochondria occur in oncocytoma, while in acinic cell cancer there are large numbers of granules in the cytoplasm Similarly, it would be of great interest to find specific structural differences which could permit a more accurate delimitation of ACC from other types of tumours, e g atypical pleomorphic adenoma Although TEM examination would not allow a clear differential diagnosis, it is of the greatest importance to collect as much information as possible on the structure of tumours

Ultrastructural studies of ACC performed on material obtained by surgery have been available for over 10 years, and have given valuable knowledge of the structures from which the tumours arise (Foote and Frazell 1954, Market 1965, Friborsky 1966, Eneroth et al 1968, Hübner et al 1969, Hoshino and Yamamoto 1970, Hamperl 1970, Tandler 1971, Engström and Stahle 1972 and Chisholm et al 1975) Most authors hold that ACC, like presumably pleomorphic adenoma (PA), originate from

the terminal ducts of the salivary gland, specifically, the intercalated ducts The majority also consider that the myoepithelial cells play a vital role Both Eneroth et al (1968) and Tandler et al (1971) have also studied the cystic structures which are found in the cribriform type of ACC, and believe them to contain material resembling a basement membrane According to Hübner et al (1969) and Eneroth (1968), these fibrillar structures are of aperiodic type, Tandler is, however, of the opinion that the content of "the pseudocysts" manifests a certain periodicity In the context of today's debate on the structure of the basement membrane, the question of its real structure should in all probability be studied more thoroughly, (Trelstad 1977)

In 1975 Chisholm et al carried out a qualitative and quantitative electron microscopic investigation of ACC in "small salivary glands" They were able to confirm that the cyst like cavities which give the tumour its characteristic cribriform appearence contain "replicated" basement membrane like material Using a stereological investigation technique they demonstrated furthermore that the cells designated ductal in the tumour occupied 75% of the volume, myoepithelial cells 3%, and acinar cells 2%, which contradicts the results of most earlier researchers, who asserted *that the myoepithelial cells predominate (Bauer and Fox 1945, Friborsky 1963,* Thackray and Lucas 1963, Hubner et al 1969) Thus opinions on the histogenesis of ACC are still divided, and justify further study

Within the framework of the investigation on TEM and aspiration cytology in head and neck tumours, we wished in this investigation to illustrate the ultrastructure of 13 cytologically certain, or highly suspect, adenoid cystic cancers and thereby compare aspiration biopsy and surgery material in terms of both quality and quantity Moreover there is the question if TEM examination of aspirates can yield more diagnostic information, than is attained by conventional cytology, which can make the pre operative diagnosis more certain

A MATERIAL

The material consists of tumours, in which the pre operative diagnosis ACC was regarded as certain (8 cases) or highly suspect (5 cases), when evaluated by conventional cytological methods Fine needle aspirates were collected for TEM examination from all these tumours Further specimens were taken post operatively in all 13 cases for light microscopy, and in 11 for electron microscopy, and compared with the pre operative aspirates In seven of these cases we also performed a semi quantitative comparison between the cell populations in material obtained pre operatively from aspirates and after operation by biopsy The semi quantitation was done on TEM micrographs

A survey of the material is given in Table III, which also illustrates agreement or disagreement in the classification of tumours by different techniques

CASE NO	ORIGIN	DIAGNOSIS		TEM		SEMI-THIN SECTION FINE N P	REMARKS
		FINE N PCT	HISTOPATH	Aspirates	Biopsy		
EH 163	Tongue base	ACC	ACC	+	—	+	
EH 164	Palate	ACC	ACC	+	—	+	
EH 167	Lgl met from parotid gland	ACC	ACC	+	+	—	
EH 214	Lgl met from larynx	ACC	ACC	+	+	+	
EH 220	Parotid gland	ACC	ACC	+	+	+	
EH 225	Parotid gland	ACC	ACC	+	+	+	
EH 249	Parotid gl ind	ACC	ACC	+	+	+	X ray 4000 rad
EH 255	Parotid gland	ACC	ACC	+	+	+	X ray 4000 rad
EH 223	Submand gland	Highly suspect ACC	ACC	+	+	+	
EH 230	Palate	Atypical PA alt ACC	ACC	+	+	(+)	Frozen section D PA
EH 237	Parotid gland	Atypical PA alt ACC	ACC	+	+	(+)	Frozen section D PA alt Mucoep ca
EH 182	Palate	Highly suspect ACC	Atypical monom adenoma	+	+	+	
EH 206	Parotid gland	Atypical PA alt ACC	Monomorphic adenoma	+	+	+	

Table III. Survey of material included with diagnosis as evaluated from fine needle aspiration cytology and biopsy.

Abbreviations ACC adenoid cystic cancer,, PA- pleomorphic adenoma, Monom ad monomorphic adenoma Mucoep ca — mucoepidermoid cancer

This survey suggests the presence of a discrepancy in the diagnosis of the tumours by means of the methods used To simplify the problem for discussion the material can be summarized as in Table IV

Table IV

Agreement	cytology (ACC) — histopat (ACC)		6
Highly suspect	cytology (ACC) — histopat (ACC)		3
Highly suspect	cytology (ACC) — histopat (other diagnosis)		2
X ray cases	cytology (ACC) — histopat (ACC)		2
		Total	13

B METHODS

The material was used for diagnostic purposes as indicated in the following survey

a) **Fine-needle aspirates**

1 For light microscopy material was aspirated, smeared on object glasses, air dried and stained by MGG

2 For electron microscopy (TEM) material was aspirated, fixed and embedded in Epon 812 as described in Part I D a

b) **Biopsy material** (taken at operation)

1 For light microscopy, a thin slice of tumour was fixed in formaldehyde (Histofix*), embedded in paraffin, sectioned and stained in Hematoxylin Eosin, PAS, Alcian green and Congo red

2 For TEM, small pieces of tumour material were collected, fixed, and embedded in Epon 812 as descibed in Part I D a

* Histofix (Formaldehyde in cacodylate buffer)

c) For a **semi quantitative comparison** Epon embedded material for TEM from operation material (B) and aspirates (A) were studied The aim was to make a pilot study of the tumour cell content in the two types

The material for semi quantitative comparison consists of the 7 cases shown in Tables III and IV. The cases chosen were:

3 cases with complete agreement (ACC) between cytology and histopathology (EH 167, EH 214, EH 225)

2 cases highly suspect to be ACC cytologically and clearly described as ACC histopathologically (EH 230, EH237)

2 cases highly suspect cytologically but both described histopathologically as monomorphic adenomas (EH 184, EH 206)

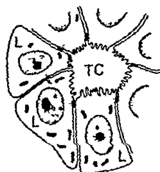
In this material 200 neighbouring cells chosen at random among TEM sections from aspirates and biopsy specimens from every case have been evaluated, counted and compared. The classification of the cells was performed partly in accordance with definitions given by Chisholm (1974) and Hoshino and Yamamoto (1970). As criteria for the present classification we have chosen the following principles where the cells are designated as, L, L_1, D, D_1 and Sq cells.

L-cells (light cells) correspond to the "duct-type cells" described by Chisholm et al. (1974). Hoshino and Yamamoto (1970) called them B-cells or "secretory type cells". The L-cells are large, ovoid or rounded. Their nuclei are often round but may be slightly serrated or cleft. The chromatin is very finely granular with condensations towards the nuclear membrane. There are often 1—2 large nucleoli, frequently with a distinct reticular nucleolonema. The cells contain but few cytoplasmic organelles in the form of mitochondria and endoplasmic reticulum. The ribosomes often appear in rosette formations, polyribosomes. The cells are interconnected by an abundance of desmosomes. Microvilli can be observed where the cells are arranged along luminal surfaces. Neighbouring cells often have interposed intercellular material although they are richly interconnected by the desmosomes.

D-cells (dark cells) correspond to the "myoepithelial cells" described by Chisholm et al. and to the A-cells as these are classified by Hoshino and Yamamoto. The structure of these cells is in many ways reminiscent of normal myoepithelial cells. They are dark in TEM-micrographs, often irregularly oblong or stellate, and smaller than the L-cells. The chromatin is abundant and densely distributed throughout the nucleus which usually has an irregular form. The cells have a rich endoplasmic reticulum and more mitochondria.

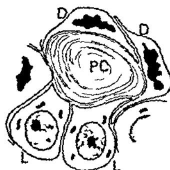
L_1- and D_1-cells correspond to the "transitional forms" described by Hoshino and Yamamoto. Similar cells were seen in pleomorphic adenomas by Eneroth and Wersäll (1966) and called intermediate forms or incompletely differentiated duct cells. They were also described as intermediate types by Welsh and Meyer (1968) and given various names by other authors.

L_1-cells (light intermediate cells) have a cytoplasm as in the L-cells but the nuclear membrane is irregular and the nuclear chromatin more clustered than in the L-cells.



PC – Pseudo cyst
Cyst-like cavities delimited by structures resem
basal membrane The cavities are normally filled
mucus like material

TC = True cyst
The cyst walls are usually delimited by "duct t
cells or L-cells with microvilli along the luminal
der



L-cells — light cells "duct-type" cells

D-cells — dark cells

L_i and D_i-cells — intermediate cells

Fig 29 Three TEM aspirate micrographs from ACC tumours showing light and dark cells true cyst and pseudocyst The schematic drawing explains the principles in the arrangement of the cells and their organelles (A 3500× B 7000× C 7000×)

D_i-cells (dark intermediate cells) have a rather dense irregular cytoplasm and a fairly well developed endoplasmic reticulum with mitochondria but the nuclei are ovoid, not as irregular and serrated as in typical D-cells

Desmosomes are not observed only in D-cells but may appear between L- and D cells and also in the intermediate forms They are most frequent between L-cells

Some authors have defined the cells in ACC as either ductal or secretory The present classification is used to avoid such a definition as it is not possible in our material to ascertain the original cell type using TEM alone It is so well known that the cytoplasm and cell nuclei in rapidly developing cell cultures or tumours may change both nuclear form and cytoplasmic density during mitosis Density of nuclei and cytoplasm alone can, however, be used in the comparison of cell content from two forms of biopsy in the same tumour

This context calls for a further definition of the so-called *pseudocysts* (PC), (Azzopardi & Smith 1959) This term denotes a cyst like formation, outwards bordered by the plasma membrane of surrounding cells Below the plasma membrane a basement like, fine fibrillar, continuous membrane surrounds the pseudocyst Several similar fine fibrillar membranes are often seen inside the pseudocysts which may also contain structures resembling cell debris Naked pyknotic nuclei can occasionally be observed in these formations The thickness of the "basement membranes" is not uniform The "true cysts" (TC), which may sometimes be ducts, consist of circumscribed cavities bordered by the L-cells which usually have microvilli on the surface facing the cysts (Fig 29)

C RESULTS

a) Cytology and PAD in complete agreement ACC — ACC
EH 163, 164, 167, 214, 220, 225, 249, 255

In these cases of complete agreement the cytological smears for light microscopic examination were rich in cells and contained numerous distinct, clearly delimited, circular or oval, mucous globules of different sizes Moreover the scrutiny revealed patches of reddish violet, homogeneous, and thread like mucus of the same type as seen in the cysts, *forming short strands between and outside the cell clusters* Where the cells were lying in isolation, open to classification, they consisted partly of large, light, vesicular cells (L-cells), partly of smaller, darker cells (D-cells) The cells inside the more compact parts of the tumour could not be classified Around the globules the inner cell layer comprised light cells rather than dark cells although usually a mixture

Corresponding histopathology revealed a tumour tissue dominated by cribriform structures with cysts of varying size and shape In one case, however, the solid areas

predominated In another, the incidence of mucus was surprisingly high, and apart from the typical cribriform structures the cysts formed elongated, ramified "duct systems" of the type often observed in ACC The cyst like structures were surrounded as a rule by one or more cell layer The cells bordering the cysts were difficult to classify but dominated by L rather than D-cells The cells were usually mixed in those cases where a differentiation was possible Where staining of the mucus with Alcian green was carried out, the content of the cysts and the tissue between the epithelial cells were fairly heavily stained The corresponding areas were only slightly stained when PAS was used Congo red staining with regard to amyloid was negative in all cases but one (EH 164) in which it was strongly positive (In this case Alcian green was only weakly positive and PAS negative)

At the corresponding ultrastructural examination fine needle aspirate revealed, apart from solid areas, an abundance of large and small pseudocysts (PC), all of which were *delimited by a distinct*, continuous line resembling a basal membrane The PC were surrounded by equal numbers of L and D-cells, the two often being intermingled Many of the cells displayed the characteristics of the L and D types, so that the nuclei were dark in colour and resembled D cells, whereas the cytoplasm had the criteria of the L or ductal cells Similarly, the nuclei could be light, of the L cell type while the cytoplasm was sparse and indented, reminiscent of the D cells in this respect Tonofilament like structures were frequently discerned in the cytoplasm, presumably an expression of squamous epithelial metaplasia

In the more solid areas the dense cell clusters often appeared to be composed of large, light L-cells with rounded, partly serrated nuclei, which were usually linked together by tightly packed desmosomes Desmosomes were much less frequent in D cells and intermediate forms

The D cells were easy to identify when they were typical, i e resembled myoepithelial cells with elongated, dark, often serrated nuclei and sparse cytoplasm containing filaments D-cells were very often found in the form of intermediate cells Similarly, a cell type suggesting metaplastic transition to squamous epithelium was often observed

The D cells were not as frequently found in large, compact clusters but lay in isolation, loosely linked with each other

Almost every specimen displayed "true" lumina (TC) which were not delimited by a membrane, but an abundance of microvilli projected into the lumina The boundary cells here consisted exclusively of the L type

The following TEM findings are also worthy of mention

One of the cases (EH 164) which was strongly positive to Congo red stain with regard to amyloid proved in TEM to consist chiefly of light L-cells, many of which contained small granules of obscure origin Since an endocrine origin could not be excluded specimens from this case were stained with silver according to Grimelius (1968) and was positive

One of our cases (EH 255) manifested a typical ACC when observed in the light microscope in both the cytologic smear and the semi thin section The TEM study

showed both L- and D-cells and pseudocysts but also a large number of cells with a very rich agglomeration of atypical mitochondria of the oncocytoma like type In this case no biopsy was made until the final operation, which took place after irradi tion of the tumour The combination of L- and D-cells with oncocytoid cells has not been observed in any other of our ACC-cases

In conclusion it can be summarized that in these eight cases the light and electron microscopic study revealed a structural arrangement of cellular structures and pseudocysts in good agreement The TEM study, however, gave a completely diffe rent picture of the internal structure of individual cells We included case EH 255 in order to demonstrate that light microscopy showed a typical ACC while the TEM study also disclosed many cells of a quite different type, full of atypical mitochon dria (Fig 31) These mitochondria were found to remain after the pre operative irradiation (4 000 rad) in the aspirated specimen No tumor cells were found in the specimen taken for biopsy after the operation while simultaneous aspirates contai ned fairly numerous cells of the oncocytoid type They had evidently been taken from different portions The TEM study also allowed a better differentiation be tween the individual cell-components of the tumour and of observation of nuclei and nucleoli as well as intercelluar contacts

b) Cytology and biopsy not in complete agreement

This group includes three cases namely

Case No	CYTOLOGY	HISTOPATHOLOGY	FROZEN SECTION
EH 230	Atypical PA alt ACC	ACC	PA
EH 237	Atypical PA alt ACC	ACC	PA alt Mucoep ca
EH 223	Highly suspected ACC	ACC	

The cytologic smears in the first two cases (EH 230 and EH 237) were rich in cells and displayed many adjacent or solid cell groups with mucoid red violet strands, but in EH 237 no typical rounded mucoid globules could be seen In EH 230 occasional, small globules were seen but not in sufficient numbers to confirm the diagnosis of ACC During operation frozen sections were studied in these two cases and as the table shows the diagnosis differed from the definite one made on paraffin sections

The TEM studies of the aspirate from EH 230 showed solid areas with L and D cells intermingled, but a clear dominance of D-cells, among which cells indicating squamous cell metaplasia were seen There were comparatively few pseudocysts and

occasional true cysts The TEM observation of this case with occurrence of PC and TC and the arrangement of L- and D cells was in good agreement with an ACC but does not with our present knowledge exclude pleomorphic adenoma

The TEM study of EH 237 demonstrated a cell structure and arrangement which differed considerably from the other cases of ACC The L-cells were atypical although the D cells fitted the above description The "L-cells" were very irregular, the nuclei displaying many indentations or clefts with pseudo inclusions The nucleoli were very prominent, the mitochondria in different stages of disintegration and there was a fairly rich endoplasmic reticulum, especially of the rough type There were true cysts with microvilli but no pseudocysts The squamous epithelium like cells contained many tonofilaments The picture obtained by examination of speci mens from the operation showed agreement with the aspirate In these sections desmosomes were also numerous between L-cells, while the aspirates demonstrated rather separated cells In this case the TEM study was not typical for ACC

In the third case (EH223) the cytologic smear contained many dark and irregular cells Red violet mucoid strands pierced rather solid groups of epithelial cells The smear contained a few PC like globules The histopathology in this case demonstra ted a solid form of ACC and the few cribriform areas contained pseudocysts surrounded by several layers of epithelial elements The TEM aspirate did not con tain a very rich material It contained L- and D cells but was especially rich in inter mediate cells There was a pronounced hyalosclerosis This is also in good agree ment with the TEM biopsy at operation which showed a very solid structure with a ılar cell content and very many intermediate dark cells There were few and .ll pseudocysts

ı conclusion this case represented a solid form and the paucity of globules in the ›logic smear made it difficult to confirm the diagnosis Electron microscopy was ood agreement with ACC

hus the investigations show that these three cases, which were hard to evaluate in ›logic smears, were also difficult to classify histopathologically, nor did they reveal a uniform picture on TEM examination The cases EH 230 and EH 223 were shown by histopathology to be a solid form of ACC with only a few cribriform structures — hence the divergent diagnosis of PA on frozen section examination of EH 230 The cytology was suspect, however, in that a few small ' mucoid" globules were found TEM examination showed typical PC structures in both cases Case EH 237, which was atypical on both histopathological and cytological examination, displayed pronounced squamous epithelial metaplasia on TEM investigation, which may explain the inaccurate frozen section diagnosis of mucoepidermoid cancer In general TEM examination of EH 237 revealed atypical ductal cells and no definite PC structures, so that the diagnosis ACC is doubtful

c) Cytology and PAD at variance

Cytology Highly suspect ACC (EH182) Atypical PA or ACC (EH 206)
PAD Monomorphic adenoma (EH182 and 206)

Cytological smears for light microscopic examination were in both cases fairly uniform being cell rich specimens in which the cells lay for the most part in thick clusters Here and there strands of reddish violet mucus pierced and enclosed the clusters In places the mucus formed small distinct globules — occurring in great numbers in case EH 182

Histopathological examination revealed a fairly similar cell picture in both cases where the epithelial cells formed confluent, primarily solid but also small tubular structures, the latter being most prominent in case 182 Mucus staining elicited both positive PAS and Alcian green staining in both cases In EH 182 no distinct tumour capsule was seen In 206 there was a breach in the fibrous capsule over a small area

The ultrastructural investigation of EH 182 demonstrated an abundance of, first and foremost, intermediate cells (both L, and D) and cells showing squamous epithelial metaplasia In addition many small PC like structures were seen usually not exceeding 1—2 cell diameters (15—30μ) The pseudocysts were surrounded by L and D-cells, the L-cells had partly cleft nuclei and contained patches of granules The L cells were firmly linked with each other, and provided with numerous desmosoms formed solid clusters The D-cells were more loosely scattered and only sparsely linked by desmosomes The TEM examination of EH 206 showed predominantly L cells but squamous epithelial metaplasia was also seen The L-cells had likewise partly cleft nuclei, containing granules here and there The L-cells seem to be less strongly linked with each other than in EH 182 with large intercellular spaces Moreover a moderate occurrence of smaller PC like structures was seen as in EH 182 Various large mucous lakes were observed, which were also surrounded by a basement membrane like structure

Thus the investigation shows that it is possible to find the mucous globules observed in the cytological smears as PC like structures in TEM These PC structures, however, could not definitely be found in paraffin sections in the form of cribriform structures Perhaps this finding in TEM may be manifestations of microadenoid cystic cancer of a type similar to that described by Evans and Cruickshank (1970) where the tumour consisted of a mixture of a microadenoid cystic cancer and malignant basal cells i e in this case it could be a monomorphic adenoma in the process of becoming a microadenoid cystic cancer

Thus no clear dominance of any cell type is seen in our tumours, at least not in the aspirate Nevertheless all tumours consist in principle of the same kind of cells, i e L, L_1, D and D together with Sq cells, and each individual tumour has its special pattern Similarly varying amounts of mucous substance are seen (see Table IV in previous chapter) with different staining properties, presumably indicating different chemical composition

e) Findings in two irradiated cases of ACC, EH 249 and EH 255

In these two cases light microscopic cytology revealed typical ACC with epithelial cells lying both isolated and in solid clusters where the cell population in the first case (EH 249) appeared to be dominated by comparatively large, round to oval cells with varying chromatic density A moderate number of much smaller cells with hyperchromatic nuclei and irregular membranes were also found The cytoplasm in the larger cells was indistinct and poorly defined In the second case (EH 255) the picture was dominated by comparatively large, light, round or oval cells, which usu ally displayed a fairly large nucleolus in the nucleus The cytoplasm was well defined and of a beautiful blue colour, in contrast to the lighter, almost indiscernible cyto plasm in the first case Both specimens presented an abundance of red violet mucus which formed distinct, small (EH 249) and large (EH 255) globules of the type seen in the other cases of ACC confirmed by PAD Moreover the mucus here and there formed structures resembling ductal casts Semi thin sections of the Epon embed ded aspirates were obtained in sufficient quantity from EH 249 before irradiation and after irradiation with 2 000 and 4 000 rad (Fig 30A) From EH 255 material was taken before and after irradiation with 1 000 2 000 and 4 000 rad (Fig 31A)

TEM examination of the material before irradiation showed typical ACC structu res with pseudocysts (Fig 30B and 31B) After irradiation with 1 000 and 2 000 rad a certain general dispersion appeared most pronounced in EH 249 The tumour cells were dispersed surrounded by an amorphous, unstructured material, probably corresponding to the reddish violet mucus seen in light microscopic smears from the same cases

After irradiation with 4 000 rad the dispersion in EH 249 increased even further, as shown by Fig 30 C and D On TEM examination PC structures could still be observed but the membrane began to lose its sharpness and the cytoplasm of the cells showed signs of degeneration The nuclei were frequently dispersed and indis tinct

Although the light microscopy showed a typical ACC picture with pseudocysts in case EH 255, the TEM study of material obtained before irradiation demonstrated a

Important Since the material so often consists of small fragments the greatest caution must be observed when rinsing and changing different fixatives. It is advisable to use a Pasteur pipette with an external diameter of only 0.5 mm, which can be prepared by drawing a glass tube. This device prevents the small fragments from being sucked into the pipette and lost.

The cells and fragments were sometimes stirred up during one of the steps in the manipulation, and the specimen was then gently centrifuged.

c) Sectioning

The specimens were first studied under a preparation microscope. Sections (1—3μ thick) from regions of particular interest were made on an LKB ultrotome. These sections were mounted on glass slides and stained with 1% paraphenylene diamine in water, or with 1% solution of toluidine blue in 1% borax solution.

For electron microscopy (TEM) thin sections were made with a diamond knife. They were stained with 5% uranyl acetate in redistilled water for 20 min at 55°C, and subsequently with Reynold's lead solution (Reynold 1963) for three min. A Siemens Elmiscop I A and a Philips 301 transmission electron microscope were used for this study.

E. RESULTS OF MATERIAL AND METHODS

The studies made on animals and human material clearly demonstrated that representative material of sufficient quantity and good quality could generally be obtained. In some cases the quantitative factor is of great interest, especially if it is difficult to obtain sufficient material. We therefore devoted considerable time to a study of needle design and aspiration technique.

The experiments with different types of needle (A, B, C, D and E) (Fig. 1 D), indicated that the shape of the needle is of interest, especially with regard to the grinding of the point, and the diameter and length of the needle.

When making aspirates from "difficult" material (kidney) we obtained different amounts of material with all types of needle. The different needles were compared, the conventional 0.7 mm injection needle being the basis of reference.

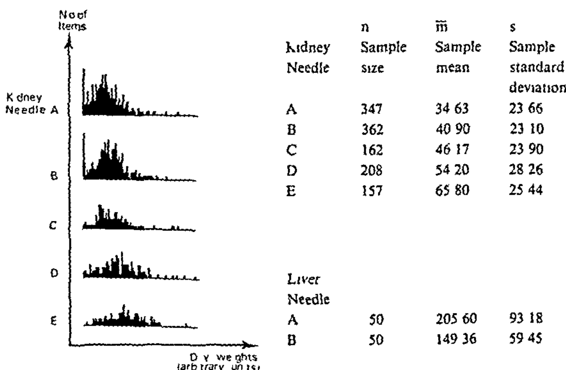
This reference needle, A, which we use for daily routine punctures to obtain smears for light microscopy, gave the poorest (least) result. Needle B, which is identical with A save that one side of the open tip has been ground to a cutting edge, gave 18% more material. Needle C, which has the same diameter (0.7 mm) as A and B but has an opening ground lengthwise (subcutaneous needle), improved on A by 35%. Needle D, which is ground like C, i.e. is a subcutaneous needle ground lengthwise but 0.8 mm in diameter, yielded 58% more than A. Needle E is an injection needle, 0.8 mm in diameter and 80 mm long, ground at the opening like C and D. This gave 100% more than A, i.e. twice as much yield (on average) per puncture. From "easily" aspirated material (liver) we obtained sufficient material for TEM,

irrespective of the type of needle used Consequently only a few punctures were made with A and B on liver If the yield from these two needles is assessed on easy and on difficult material then the needle gave five times as high a yield from the easy as from difficult material (kidney) See histogram (Table II)

The small core and fragments of tumour tissue obtained by aspiration are well suited for fixation, and very high quality is often found With such small quantities of material it is important to exclude every possibility of contamination We believe that *the use of a single tube* throughout the handling of the specimens constitutes a guarantee against contamination This precaution also involves little risk of losing important material during processing

With the method used it may happen that the glass tube does not break spontaneously, or on immersion in cold water after removal from the oven The tube can then be broken by compression in a small vice

There is a possibility that even better quality could be attained by changing the tube, but, as this could mean loss of material or contamination, we decided on the technique described, and use it daily with good results



Kidney Needle	n Sample size	m̄ Sample mean	s Sample standard deviation
A	347	34 63	23 66
B	362	40 90	23 10
C	162	46 17	23 90
D	208	54 20	28 26
E	157	65 80	25 44
Liver Needle			
A	50	205 60	93 18
B	50	149 36	59 45

Table II
Frequency distribution of dry weights of aspirates obtained by different needles

dominance of rather large cells resembling L-cells, almost all of which contained considerable amounts of densely packed mitochondria of a type often seen in onco cytoid tumours We never before observed this agglomeration of mitochondria in cases of ACC The cristae of the mitochondria do, however, differ slightly from what we generally find in oncocytomas, and are partly of the tubular form (Fig 31 C and D) PC s being numerous The finding of these agglomerations of mitochond ria with atypical cristae is not from our point of view in full agreement with the light microscopic diagnosis and a metastatic process must be considered

Even after irradiation with 4 000 rad many cells were still present and the mito chondria remained little changed Moreover PC s were present In this case biopsy material did not reveal any remaining tumour cells, evidently because different regions were studied

These two cases are of interest from different points of view Under light micro- scopy they are very similar and the diagnosis was ACC TEM demonstrated conside rable differences, however, and indicated in one case the possibility of some other kind of tumour Furthermore the repeated aspirates from the tumours during treat ment gave information concerning the effect of the irradiation These preliminary observations thus stimulated us to further studies, now in progress, of the effect of irradiation

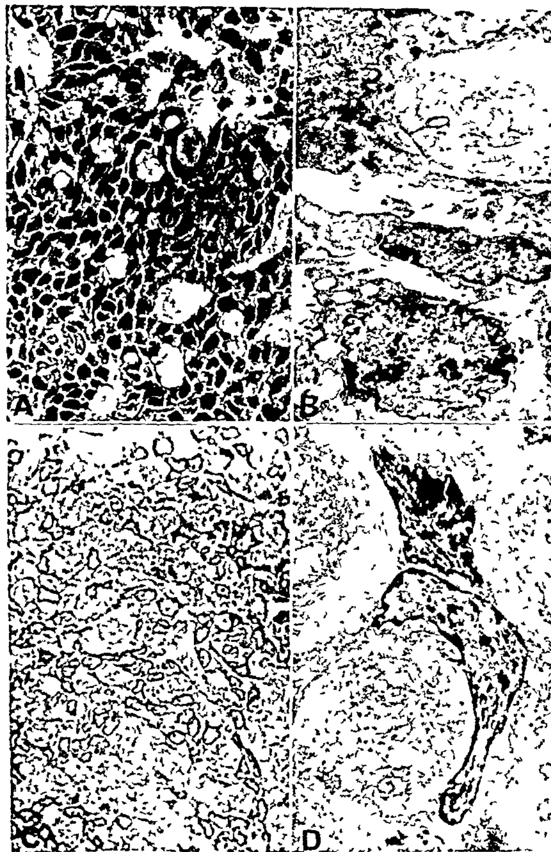


Fig 30 In this figure A and B dep t he structure of an ACC before irrad ation A and C are semi thin sections and B and D TEM I n be een how the normal texture of A is mod fied in C with reduction of cells per square surface a a e mu us In B t can be seen how the PC-conf gurat on is d stinct while D shows indistinct b d a d er a n mod f cation of nuclear structure (A 600 × B 8000 × C 600 × D 8000 ×) C and D a e ad at o 4 00 rad

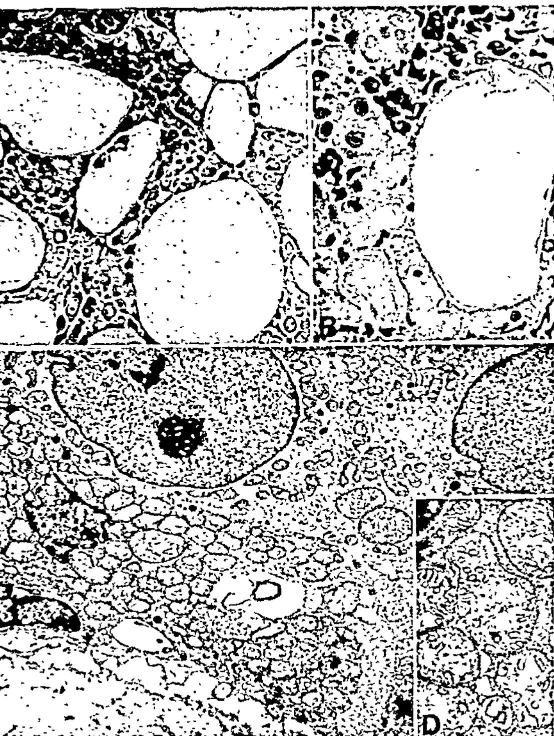


Fig 31 Adenoid cystic cancer (Case EH 255) In this controversial case the micrographs A-D demonstrate the morphology of the tumour cells The cytoplasm contains large number of atypical mitochondria ...), B is after ... (A 200 ×,

Part IV GENERAL DISCUSSION

Fine needle biopsy has become an important tool widely used in Sweden and many other countries for the diagnosis of tumours The technique is based in principle upon aspiration of tumour material by mean of a specially designed syringe and a thin needle The aspirated material is spread on an object glass, stained and studied with the help of microscopy Such a study can provide vital information about the type of tumour present Fine needle biopsy has proved to be of great value parti cularly in tumours not easily accessible for conventional biopsy Indeed in salivary gland tumours, much discussed in the present publication, fine needle aspiration has been our standard pre operative form of biopsy for more than a decade A series of publications have already demonstrated the great value and slight risk inherent in this method

In clinical practice postoperative studies of the histopathology of extirpated tumours have been constantly used and trusted in the final diagnosis of the tumours under discussion Pre and postoperative diagnosis have been clearly shown to yield results in very close agreement

When publications repeatedly appeared, reporting on transmission electron mic roscopy (TEM) studies of the material obtained by conventional biopsy it seemed natural to investigate whether TEM could also be used in the examination of the ma terial derived from fine needle biopsy In 1973 Hagelqvist and Engström published material clearly indicating that TEM studies of aspirated material could also provide further information on tumours The preliminary paper pointed out that several parameters involved in the handling of the material were of importance for the qua lity of the results During the years following this early paper the material here published was obtained and several of these parameters examined Some of the problems inherent in such a study are described in the Introduction Various major issues concern the quality and quantity of the material obtained, together with the possibility that TEM can provide important information in addition to that derived from light microscopy We are convinced that this is the case

It must be pointed out, however that many factors which have become extremely interesting for the structural analysis of tumour cells, such as mitochondria, endo plasmic reticulum cell borders and cytoplasmic inclusions of various kinds are diffi cult to study in detail with light microscopy while TEM with its higher resolution immediately distinguishes between e g cytoplasmic secretory granules and mito chondria Transmission electron microscopy also allows detailed observation of particles such as viruses or study of cell borders and cell junctions Every study demanding high resolution immediately raises the question of how the specimens should be treated to give good quality

Another factor may be very inportant in clinical work, namely the need to produce good quality within a short time. As described in the previous chapters we approached several of these problems in animal experiments and in studies on human material. Diverse other questions which we consider relevant to our work are described in the Introduction.

In animal experiments we first made aspirations from liver and salivary glands, both of which have a well known structure It became very clear that distinct structural details could be observed, and the method gave a TEM picture in good agreement with the standard picture of both liver and salivary gland cells Later studies of human material confirmed our animal results The animal experiments were continued in a study of problems related to the quantity of material obtained by fine needle aspiration The background of this study was obvious In 20 years of work on fine needle aspiration cytology we have learnt that certain tumours may yield large numbers of cells while other tumours produce but few. We therefore studied material from liver, which we regarded as an "easy" material, and compared the number of cells with material from kidneys which we considered "difficult". Liver obviously gave aspirates much richer in cell content We therefore started to investigate different kinds of needles to be used in specimen collection from different kinds of tumours We feel that special grinding of the needle opening allows us to obtain a richer material than with ordinary needles, and believe that different types of needle must be used for the best possible results We concentrated on liver and kidney material and a statistical analysis of our results indicates that the amount obtained from aspiration with specially constructed needles can be richer than material collected by conventional needles of the same diameter Nevertheless other factors such as the risk of haemorrhage and trauma must be taken into account when new needles are developed We also compared e g needle length and needle diameter and discovered that an ordinary 0 8 mm diameter needle gives more material than an 0 7 mm specially ground needle On the other hand, it seems to cause more bleeding so that we prefer the thinner needle

We also studied the importance of the length and found that a longer needle does not necessarily give more material than a shorter Our studies merely indicate the importance of the design of the needle We are continuously studying this factor which we believe to be important for certain types of tumour In the present investigation we based our observations on a comparison of dry weights of material from different kinds of needles It is not easy clearly to define the weight of the material, since contamination with blood and other fluids may confuse the results To reduce the risk of excess blood in those cases where sharp or coarse needles were used, we carried out our measurements on material freshly removed from animals We are well aware that this question can be much more carefully analysed but we used our results as a pilot study

Using animal and human material we have examined fundamental problems encountered in the preparation of aspirates for TEM-studies The factors concerned handling of the specimens, prevention of contamination and delay, compared with

other methods We used two different methods for fixation and embedding, a standard technique for ordinary cases and a rapid method for those cases in which a rapid diagnosis is important Both methods are based upon techniques commonly used in TEM studies but adapted to our needs To prevent contamination and loss of material all handling of the specimen from fixation to embedding in Epon, takes place in a single jar — a thin glass tube The time span from aspiration to electron microscopic observation can be reduced to 5—6 hours with this rapid technique We regard this time as satisfactory in most cases With this technique we have for instance been able to show within five hours after aspiration the presence of viruses

Another factor inherent in the technique was the great importance of selecting appropriate areas for TEM studies by a scrutiny of large semi thin (1—2μ) sections When these were stained we found that the large semi thin sections often provided information on the arrangement of the cells, the cell nuclei etc These sections thus had a twofold value both for selection of a special specimen area and for information on the structure and arrangement of the tumour cells

The human material used in this publication comes mainly from head and neck tumours, especially salivary gland tumours The material of about 300 tumours and inflammatory processes was studied with the help of different techniques As already discussed, we used both conventional techniques for cytological smears and biopsy and TEM In the present study less attention was paid to the differential diagnoses of the tumours or their exact classification than to the possibilities obtaining information on the tumour cells exceeding that procurable by light microscopy It is our firm belief that the intracytoplasmatic structure of tumour cells the size and shape of their nuclei and their cell borders are of importance for our understanding of the tumours and presumably for their classification in the future

In the differentiation between different intracytoplasmic structures such as secretory granules and mitochondria, light microscopy may often present certain difficulties while TEM affords immediate separation TEM may also provide important information on the individual form or structure of, for instance, mitochondria and on several occasions we were given essential assistance in the diagnosis by the structure of the mitochondria One of these cases was especially interesting A woman with tumour in her left parotid gland was operated The diagnosis was acinic cell cancer After some time she rather suddenly manifested tumours in the parotid gland on the other side and operation once again revealed acinic cell cancer The specimens were studied by several pathologists and the diagnosis was unanimous Because of the findings at TEM cytology which showed a structure differing from that of an acinic cell cancer, which normally has large numbers of secretory granules, the diagnosis was questioned The aspirated material showed an uncommon form of mitochondria (Fig 21 B D) which greatly resembled mitochondria found by Seljelid & Ericsson (1965) in renal carcinomas An investigation showed bilateral renal tumours the diagnosis being confirmed by angiography and fine needle biopsy

Other cytoplasmic structures may be much easier to discern by TEM than by light microscopy. This was the case in various amelanotic melanomas where TEM disclosed characteristic premelanosomes, not observed by light microscopy. As our knowledge of the structures of premelanosomes is not yet sufficient, difficulties may arise even with TEM. In a case of undifferentiated cancer in the parotid region with rapid invasion of cranial nerves and meninges the light microscopic aspiration cytology suggested malignant melanoma as a possibility, but the pathologists characterized the tumour as undifferentiated carcinoma. As can be seen in Fig. 26 E the tumour contains crystalloid structures with a certain resemblance to premelanosomes. The course of the patient's disease is in close agreement with either of the two possible diagnoses.

The present TEM study has clearly demonstrated the possibility of obtaining cell complexes containing many well fixed tumour cells in relation to each other and of studying the interior of the cells at high resolution. Many factors are of particular interest. Thus the cell surface and the cell borders or cell junctions can be well observed and depicted.

During the fairly short period in which the fine needle aspirates have been used the opposition to the method has been based on the assumption that the small amount of material obtained by means of aspiration would not be representative of the tumour as a whole. This is contradicted, however, by the good results, in term of diagnosis, reached by many investigators. The increased number of laboratories using the techniqe have demonstrated the close agreement between pre operative cytology and histopathology on ordinary biopsy material (Eneroth *et al.* 1967 for salivary gland tumours, Lundqvist 1971 for liver biopsies). Since the TEM investigation following fine needle aspiration is based upon the same kind of material the material could be expected to have a corresponding representativity. It could of course be said that the TEM specimen is only one small portion of the aspirate but this is also true of a biopsy specimen used for TEM. The semi thin sections are therefore of great interest, as they show many more cells and permit a localization to interesting areas. The semiquantitative study carried out on tumours of ACC-type, described in Part III, clearly demonstrates that all kinds of cells found at histopathology are also discovered at TEM-cytology. This study also indicates that the aspirate contains fewer cells with many desmosomes (L-cells) and more with few junctions (D-cells) than the biopsy specimen. This could of course be expected as highly attached cells will have a lower tendency to contribute to the aspirate. It is possible that a small biopsy can be less representative than an aspirate which includes cell groups from several parts of, for instance, a lymph node.

In ACC it is typical that mucous globules, which are very important for a light microscopic diagnosis, usually are very well represented as pseudocysts on TEM observation.

Several authors have indicated that the underpressure used at aspiration could cause cell damage. This is refused by viability-studies made by Criborn et al. (1964), and studies on imprints versus aspirates performed by Stenqvist et al. (1977).

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Part V SUMMARY

Twohundred and ninety cases of "tumours" from the head and neck region were studied in the present investigation A large proportion (160) consists of salivary gland tumours, but the material also contains various cases with infections, sialosis, mycosis, metastatic nodes, a myeloma, one eosinophilic granuloma, three malignant melanomas etc In these cases cytology and light microscopy were combined with transmission electron (TEM) according to methods described

A large proportion of the thesis is devoted to a description of the techniques used and their possibilities It is stressed that these techniques can be improved

Various questions were posed in the introduction and we believe that the results permit us to state

that TEM studies on material obtained by fine needle aspiration can be carried out with good results as judged in qualitative terms,

that the TEM aspirates generally contain sufficient material for a representative evaluation of the different cellular components of a tumour,

that the TEM micrographs provide important information on the individual cells and their organelles, which is often so valuable as to give essential clues for clinical diagnosis,

that in certain cases TEM studies can easily distinguish types of tumours which may be difficult to differentiate in light microscopy A typical example is acinic cell cancer versus oncocytoma,

that TEM observations of a specimen can if necessary be carried out, with a special technique, within 5—6 hours after aspiration,

that since aspiration of specimens can be repeated several times with little risk or discomfort for the patient, TEM studies can be used to follow and evaluate the results of a treatment using irradiation or cytotoxic drugs,

that TEM studies on aspirates should be further used to obtain a better opinion concerning the value of the method from a diagnostic point of view We believe that such studies could make a major contribution to our knowledge of various types of tumours and of their cellular components The extent to which TEM studies will eventually contribute to a better classification of tumour groups is still difficult to define but in our studies we have found TEM made on aspirates to be an important adjunct to other methods used in the pre operative classification of tumours

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ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 355

The mucociliary activity of the upper respiratory tract

III. A functional and morphological study on human and animal material with special reference to maxillary sinus diseases

by

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Introduction

Ulcerations and squamous epithelial metaplasia have both been reported in conjunction with chronic inflammation of the human maxillary sinus (Heerup & Kettel 1937, Bauer 1960, Moesner *et al* 1974) Retardation of ciliary activity might reasonably therefore be expected even before morphologically notable alterations are manifest However, no obvious impairment has been documented neither from visual observation of the ciliary activity in infected sinuses, nor from recordings of the ciliary beat frequency on biopsy material (Proetz 1933, Messerklinger 1966, Dalhamn *et al* 1959) Apparatus has been developed for recording experimentally and clinically local mucociliary activity on mucous membranes (Reimer & Toremalm 1978)

The aim of the present investigation was to elucidate functional and morphological changes in patients with chronic sinus diseases with special reference to the following questions

1 Is the mucociliary activity reduced *in vivo* or *in vitro*?
2 Is the mucociliary activity on diseased sinus mucosa different from that elsewhere in the upper respiratory tract or on normal animal reference material?
3 Can visual observations and/or physical recordings of the mucociliary activity be related to morphology?

Methods

Functional examinations

The mucociliary wave frequency was recorded indirectly via surface light reflections by a photo electric method (Reimer *et al* 1977) Most *in vitro* examinations were performed in an experimental chamber in an atmosphere of 37°C and >80 % r h The specimen was placed on a cotton bed soaked in Ringer's solution Polyps were rinsed in a specially designed chamber Comparative *in vivo/in vitro* examinations were restricted to mucosa from the posterior wall of the sinuses for technical reasons Mucociliary wave frequencies are expressed in waves/minute *In vivo* values are based on 5-10 seconds of the recordings and *in vitro* values on 1-3 recordings of 20 seconds' duration Examination time for each specimen was less than 15 minutes (Reimer & Toremalm 1978)

Morphological examinations

Specimens for ordinary light microscopy were fixed in 10 % formaline, embedded in paraffin and then sectioned and stained with hematoxylin and eosin in the standard manner Reduction of the ciliated epithelium was judged after inspection of several sections from each specimen (Table I)

Specimens for scanning electron microscopy (SEM) were fixed in 2 5 % glutaraldehyd in 0 2 M cacodylat buffer (pH=7 4) at +4°C for two hours The specimens were then repeatedly rinsed in the buffer after which they were dehydrated in acetone using the critical point method with carbon dioxide (Andersson 1951)

Finally, the preparations were coated with gold palladium in a vacuum evaporator prior to examination in a Cambridge Stereoscan MARK II A scanning electron microscope

Material

I. Antral mucosa

Twelve patients with various diseases of the maxillary antrum were investigated (Table I) The material included one case of benign retromaxillary tumor (chondromyxoid fibroma) two cases of benign mucosal cyst and nine cases of chronic sinusitis (including two with dental origin) The indication for radical operation was based on case history and X-ray examination in three projections All operations but one (VN) were performed under general anesthesia (Halothane®) and orotracheal intubation

The character (purulent or mucous) and quantity of secretions were judged visually Smears were taken during the operation and cultivated on ordinary agar medium at 37°C In five cases mucociliary activity was recorded *in vivo* from the posterior wall of the antrum After cautious excision the same part of the mucosa was then

examined *in vitro*. In a few cases several specimens from the sinus were examined *in vitro* (Table II). The functional recording was combined with morphological examinations of the same or an adjacent part of the specimen.

II. Adenoids

Epipharyngeal adenoids were removed from children aged 2-10 years. The indication was nasal obstruction and/or middle ear disease. Sixteen cases were examined. The operation was performed during a short inhalation anesthesia (Halothane®). One or several pieces of each adenoid were inspected through the operating microscope, and areas with mucociliary activity were analyzed (Table III). Several specimens were prepared for SEM.

III. Nasal polyps

Nasal polyps from 20 patients were examined. The polyps were removed after local deposition of a lidocain-adrenaline solution. Mucociliary activity could be recorded in twelve cases. Recordings were made from the same area before and after 10 minutes' rinsing with Ringer's solution in five cases (Table IV).

IV. Rabbit trachea

Twenty healthy rabbits weighing 2-4 kg were killed with a blow on the head in order to avoid pharmacological side effects. The trachea was dissected and a piece of about 5×10 mm was placed in the experimental chamber (Table V).

Table I

Patient	Diagnosis	Secretion mucous	Secretion purulent	Microbiology	Reduction of ciliated epithelium
A M A ♀ 53	retromaxill tumour	O	O		none
B A ♀ 20	*mucosal cyst*	O	O		*none (cyst)*
S B O ♀ 24	mucosal cyst	+	O		moderate (cyst) extensive (wall)
R O ♀ 58	chronic sinusitis	+ +	O	no growth	slight
G N ♂ 46	chronic sinusitis	+ +	O	staph.alb α strepto	none
H S ♂ 59	chronic sinusitis	+	O	staph.alb α strepto	moderate
S B ♂ 68	chronic sinusitis	O	+ +	α strepto	slight
A M I ♀ 50	chronic sinusitis	O	+ +	pneumococc	slight
M C ♂ 45	chronic sinusitis	O	+ +	coliform rods Aspergillus niger	none
V N ♀ 71	chronic sinusitis	O	+ +	staph aureus	slight
S R ♂ 34	dental empyema	O	+ +	staph alb α strepto	moderate
I K ♀ 48	dental empyema	O	+ +	staph.alb α strepto	extensive

O = none, + = slight
+ + = considerable

Results

I. Antral mucosa

Diagnoses and clinical findings are shown in Tables I and II The five cases recorded *in vivo* and *in vitro* and three further cases of special interest are illustrated with X-ray pictures, examples of morphological pictures and functional recordings (Figs 1-8) The mean specimen frequency varied between 784-1 599 waves/min In one case of dental empyema mucociliary activity could not be recognized at all (IK, Fig 8) In all other cases the surface of specimens looked more or less active Even in cases with purulent infections the recorded frequencies did not deviate from the other cases, but SEM sometimes revealed a quantitative reduction of cilia Details are given in figure texts

II. Adenoids

Functional results and morphological examples are shown in Table III and Fig 9 respectively The mucociliary wave frequency varied between

Table II

ANTRAL MUCOSA
MUCOCILIARY ACTIVITY (waves/min)

CASE	IN VIVO		IN VITRO		
	Analysis time (sec)	Mean frequency	Analysis time (sec)	Mean frequency	Frequency range
A M A	10	912	3×20	1208	1191—1233
B A	—	—	3×20	974	900—1101
			3×20	831	708—918
S B O	—	(516)	—	0	—
	—	—	3×20	1599	1434—1683
R O	7	934	3×20	1222	1203—1248
G N	—	—	3×20	784	750—804
H S	5	1272	3×20	1291	1233—1374
S B	—	—	3×20	1179	1104—1274
A M I	—	—	3×20	806	720—900
M C	5	1120	3×20	1252	1062—1548
V N	—	—	20	996	—
	—	—	20	1362	—
S R	—	—	20	900	—
	—	—	2×20	969	930—1008
	—	—	20	804	—
I K	—	—	several specimens	0	—

range 784—1599
mean 1078
SD 243 95

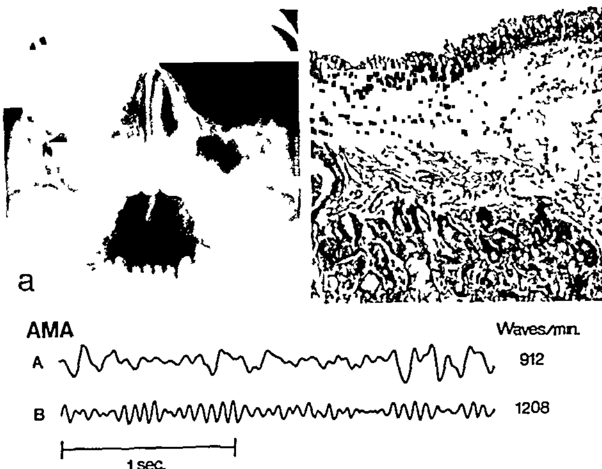


Fig 1 AMA ♀ 53 years Left sided facial pain Operation revealed a retromaxillary tumor (chondromyxoid fibroma)
a X ray picture showing haziness of the left antrum
b Photomicrograph of a mucosal specimen with a single layer of pseudostratified ciliated columnar epithelium with a scattering of goblet cells Sero-mucinous glands are seen in a slightly thickened submucosa (×40)
Below Recordings of mucociliary activity *in vivo* (A) and *in vitro* (B)

639 and 1 259 waves/minute The microscopical view of specimens with intact mucociliary activity usually showed a well preserved and dense carpet of cilia (Fig 9 a and b) In some specimens with no observable mucociliary activity a surface with some scanty groups of ciliated cells was seen (Fig 9 c and d) but in other cases there was a squamous epithelium (Fig 9 e and f)

III Nasal polyps

In twelve of twenty specimens mucociliary activity could be observed mainly on the proximal parts (Table IV) This varied between 900 and 1 296 waves/minute The effect of rinsing the specimens with Ringer s solution was varied but no dramatic frequency changes were seen (cases 1 5)

IV. Rabbit trachea

All specimens showed an intact carpet of cilia with active mucociliary activity The mean of three recordings for each specimen shows a fairly even distribution in the total material (Table V) The frequency of some specimens varied greatly even during this short period of recording (15 min)

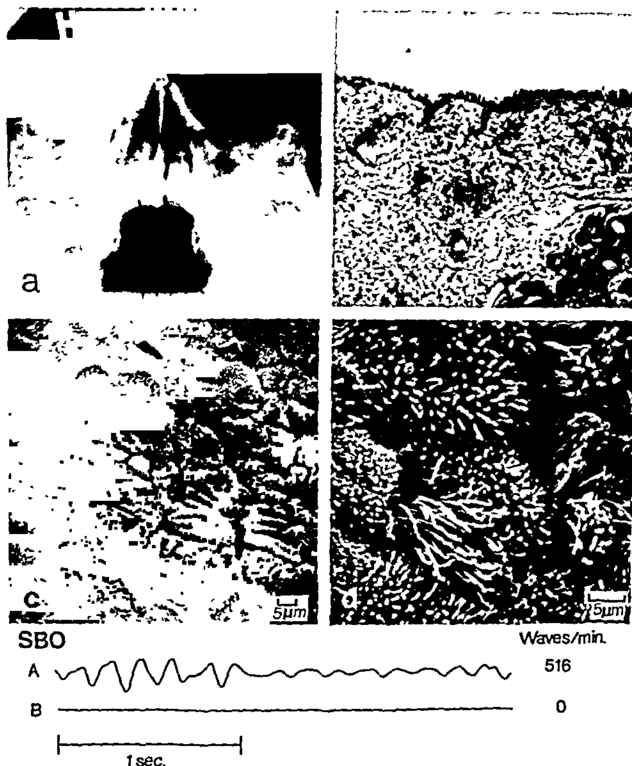


Fig 2 SBO ♀ 24 years Left sided facial pain A mucosal cyst occupied left antrum
a X-ray shows an opacified left antrum
[illegible]
(×40)
c SEM shows cells with microvilli and only a few ciliated cells (×1200)
d The cilia appear normal in higher magnification (×2400)
Below *In vivo* recording from a mucosal surface with no visible activity Recorded wave movements only represent extraneous disturbances (A) *In vitro* recording confirms absence of mucociliary wave movements (B)





Fig 2 (cont)
e Photomicrograph of the mucosal cyst A regular pseudostratified ciliated columnar epithelium with scattered goblet cells Oedema of the submucosa (×40) *In vitro* recording of mucociliary activity from mucosal cyst.





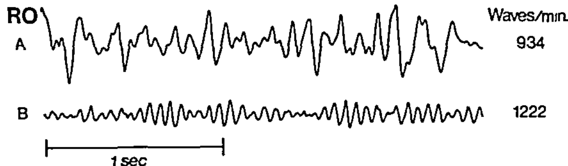


Fig 3 RO ♀ 58 years Recurrent periods of longstanding sinus infections on the right side
a X ray appearance on left side after radical operation some years previously Marked swelling of mucosa in right antrum

b Polypous mucosa with partly eroded epithelium but elsewhere normal appearing pseudostratified columnar epithelium Moderate oedema with few inflammatory cells in submucosa (×40)
Below Recordings of mucociliary activity *in vivo* (A) and *in vitro* (B)

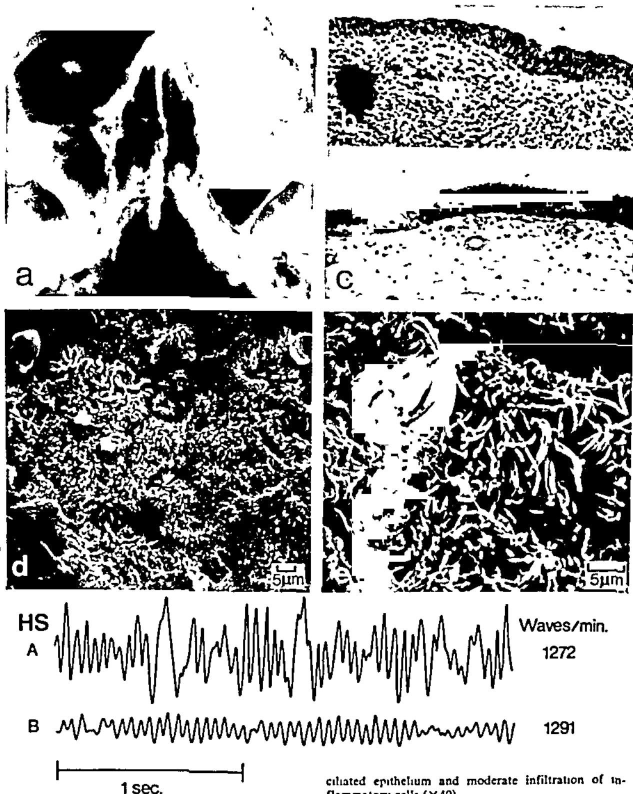


Fig 4 HS ♂ 59 years Facial pain on right side for six months
a X-ray shows opaque right antrum (note artificial eye on this side)
b Mucosal specimens of varying appearance Low ciliated epithelium and moderate infiltration of inflammatory cells (×40)
c Higher epithelium and oedema of submucosa 40)
d e SEM reveals marked reduction of cili irregularly arranged and slightly deform e×2400)
Below Recordings of mucociliary a and *in vitro* (B)

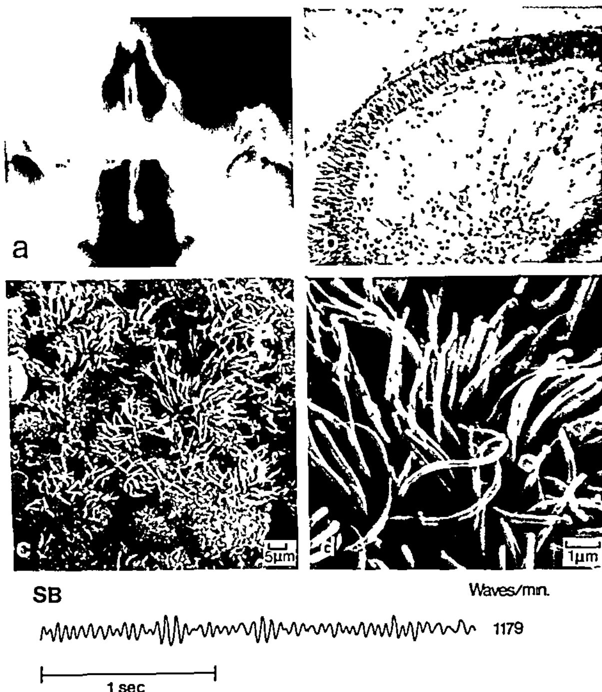


Fig 5 SB ♂ 68 years Recurrent nasal polyps and chronic purulent sinusitis on the left side
a X ray shows an opacified left antrum
b Mucosa covered by regular pseudostratified ciliated epithelium Oedematous and thickened submucosa with slight infiltration of inflammatory cells (×40)
c SEM shows quantitative reduction of ciliated cells Nonciliated cells are covered by microvilli
d Individual cilia appear normal in higher magnification (c×1200 d×12000)

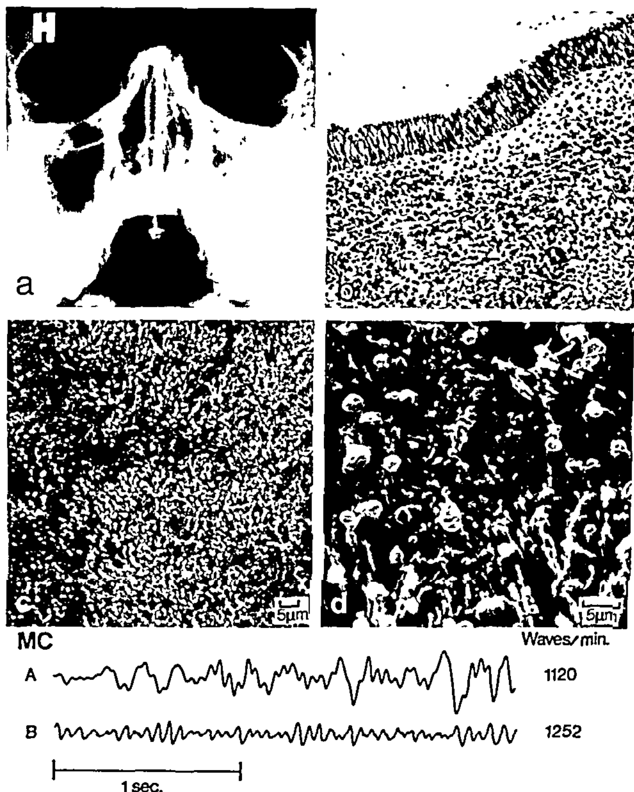


Fig 6 MC ♂ 45 years. *Purulent chronic sinusitis on left side*

a Opacified left antrum in x ray examination

b Heavy infiltration of inflammatory cells in submucosa, which is covered with pseudostratified columnar epithelium of normal appearance

c. *SEM shows a dense carpet of cilia*

d In higher magnification it is seen that the tips of several cilia are deformed (c × 1200, d × 2400)

Below Recordings of mucociliary activity *in vivo* (A) and *in vitro* (B)

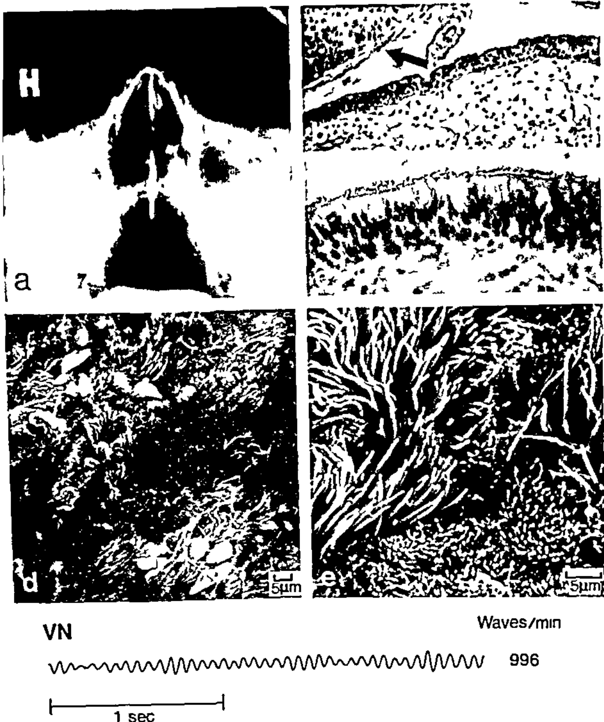


Fig 7 VN ♀ 71 years Right sided purulent nasal discharge for 6 months
a X ray reveals completely opaque right antrum
b Varying appearance of the epithelial lining Many columnar cells have vanished and remaining cells take on a rounded form A part with squamous metaplasia is also seen (arrow)
c Another part of the same specimen Regular columnar ciliated epithelium (b×40 c×600)
d e SEM shows quantitative reduction of cilia In higher magnification the cilia look rather normal d×1200 e×2400)
Below Recording of regular mucociliary activity *in vitro*

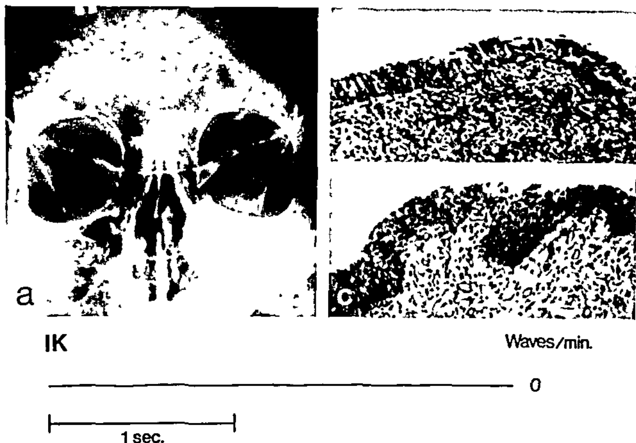


Fig 8 IK ♀ 48 years Left-sided dental empyema
a Completely opaque left antrum on X-ray
b c. Occasional areas with ciliated columnar cells but remaining parts eroded Other parts transformed to squamous epithelium (×40)

Table III

ADENOIDS

CASE	MUCOCILIARY ACTIVITY (waves/min)		
	Analysis time (sec)	Mean frequency	Frequency range
1 RG	20	1176	—
	20	936	—
2 LJ	2×20	1156	
	3×20	1226	1052—1260
			1095—1332
3 JA	3×20	1259	1194—1386
4 AP	3×20	639	553—800
5 PM	3×20	879	850—900
6 OS	3×20	987	800—1203
7—16	several specimens		no activity

range 639—1259
mean 1032
SD 211 92

Table IV

NASAL POLYPS

CASE	MUCOCILIARY ACTIVITY (waves/min)		
	Analysis time (sec)	Mean frequency A	B
1	20	1110	1386
	20	1260	1122
	20	1098	1200
	20	906	912
	20	900	930
	20	1074	—
	20	1296	—
	20	948	—
	20	942	—
	20	1080	—
	20	990	—
	20	1194	—
—20	no activity		

range 900—1296
mean 1067
SD 134 29

first recording

10 minutes later after rinsing with Ringer s solut on

Table V

RABBIT TRACHEA

Rab bit no	MUCOCILIARY ACTIVITY (waves/min)		
	Analysis time (sec)	Mean frequency	Frequency range
1	3×20	1336	1251—1452
2	3×20	1114	1065—1146
3	3×20	1028	990—1074
4	3×20	1229	1152—1284
5	3×20	1234	1036—1365
6	3×20	1048	960—1110
7	3×20	1074	969—1140
8	3×20	1034	990—1088
9	3×20	1247	1200—1296
10	3×20	1034	924—1096
11	3×20	997	984—1005
12	3×20	1389	1269—1560
13	3×20	1217	1071—1290
14	3×20	949	800—1044
15	3×20	950	720—1110
16	3×20	1363	1335—1386
17	3×20	1434	1317—1527
18	3×20	1078	993—1080
19	3×20	999	852—1136
20	3×20	1191	1092—1356

range 949—1434
mean 1145
SD 153 35

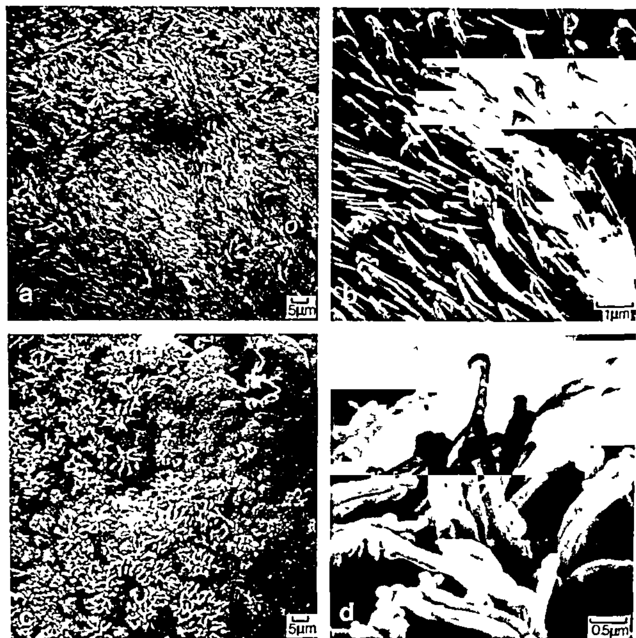


Fig 9 Examples of surface structures on adenoids
a b A case with clearly visible and recordable mucociliary activity SEM shows a dense carpet of cilia, which appear fairly normal (a × 1200, b × 12000)
c d Specimen with no visible mucociliary activity Scanty ciliated cells are seen Many of the non-ciliated cells are covered by microvilli In higher magnification it is seen that cilia are bent and slightly deformed (c × 1200, d × 24000)

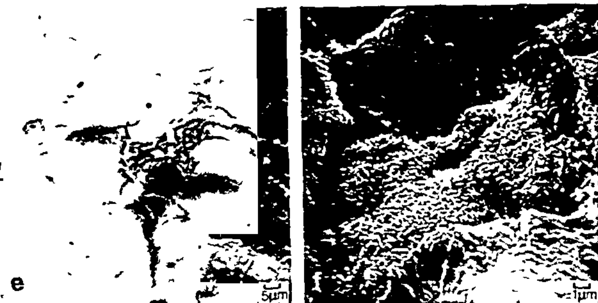


Fig 9 (cont)
e, f Another case without visible activity A squamous epithelium can be seen The cells are without cilia and actual microvilli In higher mangification some cells show an irregular surface structure (e × 1200 f × 6000)

Discussion

Retention of secretions in the paranasal sinuses may be due to several different factors (1) Swelling of the mucous membranes leading to reduced patency of the ostia, (2) overproduction of secretions, (3) quantitative reduction of cilia, retardation of ciliary movements and insufficient coordination of cilia followed by reduced transport capacity

There is evidence of vigorous ciliary activity in chronic purulent sinusitis (e g Proetz 1933) However, most reports are only based on visual observations of light reflections on mucous membranes As far as we know, nobody has compared visual observations and objective recordings of beating cilia to a clinical morphological examination of the actual epithelial surfaces We could observe and in some cases also record vigorous activity during operations On each specimen, when examined *in vitro*, certain fluctuations of activity could be seen during the examination period The *in vivo* recordings are based on relatively short periods due to methodological difficulties (Reimer & Toremalm 1978) Continuous frequency fluctuations and differences between locations (cf VN and SR in Table II), prevent an exact comparison of the *in vivo* and *in vitro* recordings, but there do not seem to be any extreme discrepancies

The supplementary investigation of the morphology of the sinus epithelium often demonstrated a well preserved pseudostratified structure with intact cilia Areas with metaplasia were usually limited but the scanning pictures revealed an obvious variation regarding the distribution of ciliated cells and the structure of individual cilia A considerable quantitative reduction of cilia was shown in some cases (SB and VN) but this finding was not apparent in corresponding light microscopy In the two cases of dental empyema there was an intense

flammatory response in the submucosa In one case the change in the epithelium was general (IK, Fig 8)

SEM investigation of adenoids was mainly performed to test if the absence of mucociliary activity, as judged by visual observation through the operating microscope, corresponded to morphological absence of cilia Adenoids are normally covered with a pseudostratified respiratory epithelium interrupted by parts with stratified squamous epithelium (Falk 1963) Most of the adenoids had no observable mucociliary activity, and the SEM examinations consistently confirmed the almost total absence of cilia In adenoids with preserved mucociliary activity a significant deviation from the mean frequency was seen only in one case (AP)

Ciliary activity failed on many nasal polyps especially on those parts which were exposed to the inspiratory air stream Analogous distribution of ciliated cells has been described in a morphological study by Mygind *et al* 1974 The rinsing of polyps did not result in any apparent change of mucociliary activity Bleeker & Hoeksema (1971) found a slow beating frequency on nasal mucosa from patients with chronic rhinitis and the activity was normalized after a similar procedure

The outcome of our rinsing experiments seems to confirm that the viscoelasticity of mucus, as a working medium for cilia, is optimal at 37°C and >80 % r h (Table IV) which is in accordance with previous experimental investigations (Mercke et al 1974 Mercke & Toremalm 1976)

For obvious reasons it is difficult to collect an adequate reference material of healthy respiratory mucosa from humans A comparison of human material from different locations may be of guidance As seen from mean values in Tables II, III and IV there is no difference between the adenoids polyps and sinus specimens in spite of their different structures It can be seen from Fig 10 that there is a wider distribution of the human specimens compared to healthy rabbit tracheas but the difference of mean values is not statistically significant

Our findings regarding the epithelium of the diseased maxillary sinus can be summarized as follows

1 The mucociliary activity can be observed and recorded from a light reflecting area through an ordinary operating microscope after removal of an excess of secretions
2 If cilia exist on the mucous membrane they have fairly normal activity even in case of chronic purulent infections
3 A quantitative reduction of cilia is sometimes seen, but they are active and the mucociliary wave frequency is rather normal
4 The frequency of the mucociliary activity is mostly about 1 000 waves/minute, which can also be found on adenoids and nasal polyps
5 The mucociliary activity is about the same *in vivo* and *in vitro*

The respiratory epithelium of the maxillary sinus is obviously markedly resistent to the physical alterations occurring in chronic sinus infections (Aust & Drettner 1974 a and b, Carenfelt & Lundberg 1978) The proposed inhibitory effect on ciliary action by lowered oxygen tension in the antrum (Flottes *et al* 1960) must be questioned, and an experimental study on this issue is in preparation

General destruction of the epithelium was in this study seen only in one case of dental empyema Probably character and number of infective organisms are of greater importance for inhibition of ciliary movements and ultimate destruction of the epithelial lining

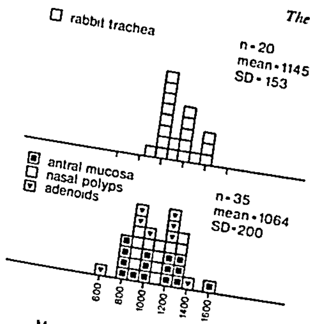


Fig 10 Mean specimen frequencies of animal and human specimens. Range of human material is wider but difference of mean values is not statistically significant. (t test, P > 0 05)

Summary

Local mucociliary activity (mucociliary wave frequency) was recorded *in vivo* and *in vitro* in maxillary sinus diseases Activity was fairly normal even in cases with chronic purulent sinusitis Scanning electron microscopy (SEM) sometimes showed a quantitative reduction of cilia The mucociliary wave frequency was mostly about 1 000 waves/min, and there was no *in vivo/in vitro* discrepancy Antral mucosa was compared to adenoids, nasal polyps and healthy rabbit trachea The mucociliary wave frequency was about the same in all materials

Zusammenfassung

Die örtliche mukoziliare Aktivität (mukoziliare Wellenfrequenz) bei Erkrankungen der Kieferhöhlen wurde in vivo und in vitro registriert Auch bei Fällen von eiternder chronischer Kieferhöhlenentzündung war die Aktivität wesentlich normal Mit elektronischer Scannermikroskopie (SEM) wurde in manchen Fällen eine quantitative Reduktion der Zilien festgestellt Die mukoziliare Wellenfrequenz betrug meistens 1 000 Wellen pro minute ohne Unterschied in vivo und in vitro Antrumschleimhaut wurde mit adenoiden Vegetationen, Nasenpolypen und gesunder Kaninchen trachea verglichen Die mukoziliare Wellenfrequenz war in sämtlichem Material etwa gleich

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Hearing in Diabetics

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Introduction

One of the most frequently occurring diseases with more or less generalized vascular pathology is diabetes mellitus (DM) Almost invariably angiopathy has been demonstrated in different organs In a few investigations the inner ear has been histologically examined in patients with DM and in all of these, inner ear changes have been demonstrated The following histological findings were demonstrated

Cochlea

Modiolus Thickening of vessel wall (Costa, 1967) Hemorrhage (Kovar, 1973)

Spiral lamina Nerve fibers decreased (Makishima & Tanaka, 1971)

Organ of Corti Hair cell loss (Makishima & Tanaka 1971)

Stria vascularis Thickening of vessel wall and atrophy (Jorgensen, 1961, Costa, 1967, Makishima & Tanaka 1971 Kovar, 1973)

Spiral ligament Thickening of vessel wall (Kovar 1973)

External spiral sulcus Debris and spherical bodies (Kovar, 1973)

Endolymph Hemorrhage (Kovar, 1973)

Perilymph Hemorrhage (Kovar 1973)

Spiral ganglion Atrophy (Makishima & Tanaka, 1971)

Internal auditory meatus Fibrous thickening of vessels (Makishima & Tanaka 1971)

Acoustic nerve Thickening of vessel wall of vasa nervorum (Jorgensen 1962, Kovar, 1973), Demyelination beading (Makishima & Tanaka 1971 Kovar 1973)

Central acoustic pathways Degeneration (Makishima & Tanaka 1971)

As a result of the generalized microangiopa in the peripheral, as well as the central, a tory system one would expect some influe from the disease on the hearing function a previous report a review of the literat was made (Axelsson & Fagerberg, 1968) T compilation showed that up to 1965 o simple hearing tests and, at the most, p tone audiometry had been performed Var degrees of hearing loss from 0 to 80% h been detected in those tested, a variat which was hard to explain No attempts h been made to determine the site of lesion the hearing loss

Since that review, additional reports ha appeared dealing with the hearing functi of diabetics These are shown in Table More modern audiological tests have be adopted but there remains a large variati in the frequency of demonstrated hearing los depending on the age of the patients, tl methods of testing and apparently on tl definition of hearing loss Many investigato have tried to correlate hearing loss and d ferent diabetic parameters These results a also shown in Table I. The only consiste positive correlation in these studies seem to be between age and hearing loss In shor reports from the last ten years confirm th findings of the previous literature revie showing that audiological test results of dia betics vary widely There is a general lack c statements as to whether group trends or in dividual cases are being reported Moreover limits have not been defined between norma and pathological hearing nor is it clea whether corrections have been made for pres bycusis, noise induced hearing loss, etc Con

Table I *Recent audiometric investigations*

TFT=tuning fork tests PTA=pure tone audiometry, SRT=speech reception threshold SD=speech discrimination DisSD=distorted speech discrimination TD=tone decay, AF=auditory fatigue, AR=acoustic reflex, Bék=Békésy audiometry DA=directional audiometry, DLI=difference limen intensity, ART=auditory recovery test

Author year	No of cases	Age	Method of examination	Hearing loss due to DM frequency
S[illegible] & Malicka 1966	32	5–40	PTA AF, TD	56%
Axe[illegible]n & Fagerberg 1968	99	20–49	TFT, PTA SD DisSD, Bék AR, DA	Group means normal —indiv analysis— few pathol findings
[illegible] & Anderson 1968	30	11–43 (median 17)	PTA, Bék	0% 57%
[illegible] et al 1969	109	10–79	PTA	44%
[illegible] et al 1969	86		PTA	21%
[illegible] Davis 1971	130	All	TFT, PTA, SRT, SD Bék	Rare—83%
[illegible] 1973	23		Bék	Yes
[illegible] 1974	664	3–75	TFT, PTA, SD	29%
[illegible] 1974	60	20–49	PTA SRT, SD DisSD TD AF, Bék, DLI, ART	Normal—73%
Friedman et al 1975	20	22–70 (median 52)	PTA	55%
M[illegible] et al 1975	124	14–65		72% mild loss

sequently, it is very difficult to compare previous results

In our previous investigation, mean hearing thresholds from patients grouped according to age were normal Few individual pathological findings due to DM exclusively could be demonstrated It appeared that audiological findings favored a retrocochlear rather than a cochlear lesion as an explanation of the hearing loss A retrocochlear involvement in diabetic hearing loss has been confirmed in other recent investigations as well (Marshak & Anderson, 1968, Rosen & Davis, 1971, Roach, 1973, Marullo, 1974)

Evaluation of the acoustic stapedius reflex has been found to be of value in the differential diagnosis of various disorders of the ear The sensation level of the stapedius reflex is "the difference in decibels between auditory threshold and acoustic reflex threshold (Jerger et al , 1974) A decrease in the sensation level of the reflex has been found in cochlear pathology (Metz, 1952, Thomsen 1955 Jerger, 1970), thus involving the afferent portion of the reflex arc An increase in reflex sensation level could reflect retrocochlear (*afferent*) involvement (Anderson et al , 1969), brain stem (*central*) involvement (Jerger et al , 1975) or facial nerve (*efferent*) involvement (Anderson & Wedenberg, 1968, Borg & Zakrisson, 1973) Absence of a measureable stapedius muscle contraction could also be interpreted as due to pathology in any of these three portions of the reflex arc (Greisen & Rasmussen, 1970, Jerger, 1970) Conductive pathology in the stimulus or probe ear can also result in an elevated or absent stapedius reflex Also of interest in the stapedius reflex response is the length of time it persists with continued tonal presentation When pure tones are presented 10 dB above stapedius reflex threshold, the reflex in normal listeners is maximally sustained for at least 10 sec at 0 5 and 1 kHz, but not at 2 and 4 kHz This reduction in amplitude of the stapedius reflex upon continued acoustic stimulation is termed stapedius reflex

Correlations between hearing loss and	No correlation between hearing loss and
Age	Sex duration DM Severity DM *Complications* DM
Duration retinopathy insulin treatment	
Age *duration* DM *microangiopathy* cerebral sclerosis	
Age duration DM retinopathy	Severity
Cardiovasc disease with DM	Age
Duration	
Age	
Duration nephropathy retinopathy neuropathy	

decay The amount of reflex decay is expressed as the number of seconds taken for the response amplitude to fatigue by 50%, and a half life time of ≤5 sec at 0.5 or 1 kHz is considered pathological (Anderson et al , 1969) *Such* abnormally rapid stapedius reflex decay at 0 5 and 1 kHz has been found in cases of N VIII pathology (Anderson et al , 1969, 1970*a b*)

The aim of the present investigation was to evaluate the general health, hearing and vision of diabetics 50 to 70 years of age, treated both orally and with insulin While our previous report dealt with the hearing in younger diabetics, this paper reports the results of all the age groups There is a special emphasis on the stapedius reflex threshold and reflex decay tests in the present study in order to elucidate the question of cochlear versus retrocochlear hearing loss

Material and Methods

The present material is given in Table II It contains 205 individuals 112 males and 93 females Mean age duration of the disease, and mean age at onset are also presented Previous data gathered on younger diabetics (Axelsson & Fagerberg 1968) are included in the present investigation The patients were not deliberately selected and constitute consecutive cases who came from two different outpatient clinics for internal medicine All patients were asked if they would take part in the examination and all patients who agreed are included in the study Thus, patients with severe diabetic complications and patients with occupational hearing loss, etc were not excluded The patients were carefully examined from the internal medical, ophthalmological and audiological points of view The examinations performed and normal values adopted are summarized in Tables III and IV

All audiometric evaluations were performed in sound proof booths with diagnostic audiometers calibrated to ISO (1964) specifications Speech reception thresholds and word discrimination data were obtained by monitored live voice or with tape recordings using spondeic and monosyllabic word lists (Liden, 1954) Speech discrimination testing was accomplished at a 30 dB sensation level or, if intolerable, at most comfortable level Contra lateral stapedius reflex threshold testing was included as part of our audiological test battery In the latter testing, the signal intensity at each test frequency (0 25, 0 5, 1, 2 and 4 kHz) was increased until the smallest definable reflex was observed or until the absence of a response was noted at 120 dB hearing

Table II *Present material*

	Total	Insulin treatment Age groups <29	30–39	40–49	50–59	60–69	Oral treatment Age groups 50–59	60–69
Number	205	34	30	40	31	26	18	36
Male/female	112/93	16/18	19/11	18/12	12/19	17/9	9/9	21/15
Mean age	Years	21 9	34 4	44 6	54 7	64 8	54 2	63 4
Mean duration DM	Years	10 3	15 3	14 6	16 6	16 3	6 1	6 7
Mean age DM onset	Years	11 6	19 1	30 0	38 1	48 5	48 1	56 7

level A few statements are necessary with regard to our judgement of pathological reflex thresholds Acoustic reflex thresholds are known to vary with age (Jepsen, 1955, Jerger, 1970 Habener & Snyder, 1974, Axelsson & Lewis 1976) This variation is evident in Fig 1 which shows normative data for stapedius reflex thresholds in dB sensation level according to age (Habener & Snyder, 1974) together with a shaded area between 70 and 95 dB representative of the normal range for stapedius reflexes in dB sensation level adapted from Peterson & Liden (1972) Since Habener & Snyder presented stapedius reflexes in dB sensation level and results were classified in terms of age, most of our computations were based on their data Reflexes exceeding ±10 dB of the means presented by Habener & Snyder (see Fig 1) were considered pathological In their normative data, except for a somewhat larger range for the 30–39 and 50–59 age groups at 4 kHz, this 20 dB range corresponded to ±3 standard deviations from their mean values (Snyder, 1977) Habener & Snyder did not present reflex threshold data for 0 25 kHz

It is mentioned that the criteria for pathology selected by Anderson & Wedenberg (1968) were pathological reflexes elicited at either 4 out of 6 frequencies at one ear or 2 out of 6 frequencies at both ears Since we tested reflexes at 5 frequencies and thresholds at 0 25 kHz were omitted because of lack of normative data our criteria for pathological reflex thresholds were reflex sensation levels outside the normal range at three out of four frequencies at one ear or at a total of three frequencies at both ears These criteria were selected as being most similar to those of Anderson & Wedenberg (1968)

Stapedius reflex decay testing was only accomplished with those patients over 50 years of age In this test, pure tones at 0 5 and 1 kHz were presented for 15 sec or more at 10 dB above the stapedius reflex thresholds and the results were recorded using a strip chart recorder If the amplitude of the response was reduced by 50% in 5 sec or less, the results were considered to be pathological (Anderson et al , 1970*a*)

The audiological results were analyzed as

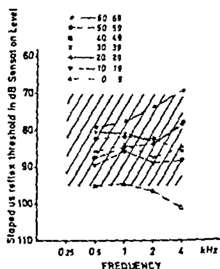


Fig 1 Stapedius reflex normative data in dB sensation level

Table III *Present material Medical examinations*

Insulin treatment Age groups					Oral treatment Age groups	
≤29	30–39	40–49	50–59	60–69	50–59	60–69
History Ophthalmoscopy[1] Kidney function test[2] Neurological examination[3a] Skin temp measurement[4] Radiology of the legs[5] Oscillometry[6] Cholesterol [7a] Histol exam skin biopsy[8]			History Ophthalmoscopy[1] Kidney function test[2] Neurological examination[3b] Occurrence of angiopathy[9] Blood pressure[10] Oscillometry[6] Cholesterol & triglycerides[7a, b]			

[1] The occurrence and degree of aneurysm hemorrhage exudate and proliferation in the retina were evaluated and graded

[2] Nephropathy = constant proteinuria without signs of infection

[3] (*a*) Includes routine clinical neurological examination electromyography electroencephalography and conduction velocity of motor nerve according to Fagerberg (1959) Neuropathy = 2 or more neurological symptoms or signs typical of DM (*b*) Includes routine clinical neurological examinations

[4] According to Brattgård et al (1951)

[5] Arterial calcifications in the lower extremities evidenced by radiology

[6] Oscillatory excursions in the ankle were measured Pathology = remarkably small (0–0 5 units) or appreciably smaller than those in the wrist

[7] *(a) Serum values of 300 mg/100 ml or above are considered abnormal* (*b*) Serum values above 2 mmol/l are considered abnormal

[8] According to Säve Söderbergh et al (1967)

[9] Evidenced by gangrene (ulcerating process in the lower extremities) and/or coronary heart disease (infarct or angina pectoris produced by physical or psychological strain and verified by ECG abnormalities)

[10] Pathology = diastolic pressure ≥110 mmHg

Table IV *Present material Audiological examination*

Insulin treatment Age groups					Oral treatment Age groups	
≤29	30–39	40–49	50–59	60–69	50–59	60–69
History ENT-examination Tuning fork tests whispered and spoken voice Pure tone audiometry[1] Speech discrimination level[2] Distorted speech audiometry[3] Stapedius reflex test[4] Directional audiometry[5] Békesy audiometry[6]			History ENT-examination Tuning fork tests whispered and spoken voice Pure tone audiometry[1] Speech discrimination level[2] Speech reception threshold Stapedius reflex test[4] Stapedius reflex decay test[7]			

[1] Normal according to age norms (Spoor 1967)

[2] ≥92% of 50 Swedish monosyllabic phonetically balanced words tested at 30 dB sensation level (Lidén 1954)

[3] According to Linden (1960 1964)

[4] According to Peterson & Lidén (1972) and Habener & Snyder (1974)

[5] According to Nordlund (1963)

[6] Normal excursions within 3–12 dB

[7] According to Anderson et al (1970*a*)

group means and as such included all results without correction for age, history, etc Individual analysis was also made In the individual analyses, the history of the patient and his age were correlated with his audiological results For instance, when a sensorineural high tone loss was found in a diabetic welder who had been working for many years in a shipyard, the loss was considered a result of noise exposure and not DM Similarly speech discrimination in elderly patients with a sensorineural hearing loss could not be expected to be normal (≥92%) In short, hearing losses were regarded as due to DM only when they could not be explained reasonably by some other etiology

In the correlative analysis all cases with pathological audiological results considered due to DM were treated as a group and compared with the whole age group with respect to ophthalmological and other medical parameters In particular the following ophthalmological and medical parameters were checked for each age group

- Heredity
- Occurrence of keto-acidosis and of insulin coma
- Occurrence of cataract
- Occurrence of aneurysm, proliferations, hemorrhage or exudates of retinal vessels
- Occurrence of amaurosis
- Occurrence of nephropathy and urinary infections
- Occurrence of neuropathy
- Occurrence of gangrene

The audiological results were also correlated to each other, e g , pure tone audiometry for individuals with abnormal stapedius reflex decay was compared to the pure tone audiometry of the respective age group

Results

PURE TONE AUDIOMETRY

The uncorrected test results are within normal ranges for the age groups as defined by *Spoor* (1967) Age-corrected group means for pure tone audiometry are shown in Fig 2 and Table V In general even after correction for age there is a slight and regular decrease of thresholds as a function of frequency with age The comparatively poor hearing at 4 kHz is due to the inclusion of many patients with noise induced hearing loss in the present study

The results of the individual analysis, in which each patient s audiogram was related to his history are presented in Table VI It can be seen that 8 kHz is the most affected frequency, and that hearing loss decreases towards the low frequencies The distribution also shows a clear correlation with age in that the incidence of pure tone hearing loss increases with age, even after correction for presbycusis (Tables VI and VII) (Spoor, 1967) There also appears to be a tendency for patients treated with insulin in the higher age groups to have better pure tone audiometry than those treated with oral anti diabetic medicine The duration of the disease in those treated with insulin was on the average 10 years longer than in those treated orally

In order to evaluate sex differences with relation to pure tone audiometry, patients with least noise exposure of both sexes were compared Females in general had poorer hearing than males In the female 8 kHz was invariably the most impaired frequency, while in three male age groups the most impaired frequency was 4 kHz It has to be emphasized

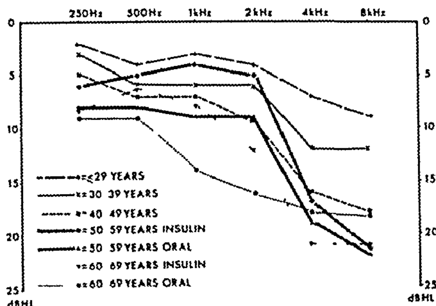


Fig 2 Hearing in diabetics Pure tone audiometry Right+left Group means

that the sex relationship is difficult to evaluate due to the higher incidence of noise exposure in the male patients

Table VIII shows parameters which were more frequent in patients with pathological pure tone audiometry corrected for age and history than in the whole age group

In summary group mean pure tone thresholds were normal for all age groups The individual analysis showed a sensorineural hearing loss due to DM in 9-31% of the subjects with incidence increasing with age high frequencies are most affected Hearing loss due to DM was more common in women than men The hearing loss may be correlated to nephropathy and severe retinopathy but not to more discrete forms of retinopathy There were no correlations of pure tone results with other audiological findings

Table V *Audiological results Group means*

	Insulin treatment Age groups					Oral treatment Age groups	
	≤29	30-39	40-49	50-59	60-69	50-59	60-69
Pure tone audiometry see Fig 1	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Speech reception threshold dB right/left	5/6	5/7	9/12	15/16	19/20	24/25	23/25
Speech discrimination % right/left	96/97	96/96	95/93	96/95	94/87	97/90	91/89
Discrimination distorted speech %	81	84	82				
Discomfort level dB right/left				94/93	97/97	96/96	94/95
Békésy audiometry excursions dB	10	13	17				
Stapedius reflex hearing level/sensation level	88/85	88/80	87/76	86/73	87/63	87/69	89/63
Stapedius reflex decay test 500 Hz seconds right/left				14/13	14/13	14/14	15/13
Stapedius reflex decay test 1 000 Hz seconds right/left				15/14	14/14	14/13	14/14

Table VI. *Pure tone audiometry. Individual analysis. Distribution by frequency for pathological ears*

	Insulin treatment Age groups					Oral treatment Age groups	
	≤29	30–39	40–49	50–59	60–69	50–59	60–69
250 Hz					2		2
500 Hz					2		2
1 000 Hz					3	1	6
2 000 Hz			1	3	5	1	6
4 000 Hz		2	4	8	8	2	11
8 000 Hz	4	5	3	12	11	4	15

Table VII. *Audiological results. Individual analysis*

DM=diabetes mellitus, PTA=pure tone audiometry, SRSL=stapedius reflex sensation level, NIPTS=noise-induced permanent threshold shift

	Insulin treatment Age groups					Oral treatment Age groups	
	≤29	30–39	40–49	50–59	60–69	50–59	60–69
Pure tone audiometry							
No of patients tested	34	30	30	31	26	18	36
No of pathological patients							
Corrected for age	3	13	12	16	19	8	10
Corrected for history	3	4	4	6	8	3	8
% pathological patients due to DM	9	13	13	19	31	17	22
Speech audiometry							
No of patients tested	34	30	30	31	26	18	36
No of pathological patients							
Corrected for PTA	10	10	9	7	6	4	7
% pathological patients due to DM	30	33	30	23	23	22	19
Stapedius reflex test							
No of patients tested	16	23	27	26	26	18	31
No of pathological patients with elevated SRSL[a]							
Corrected for conductive loss	8	0	3	1	2	0	3
% pathological patients due to DM (elevated SRSL)	50	0	11	4	8	0	10
No of pathological patients with decreased SRSL[a]							
Corrected for NIPTS	2	5	5	7	2	4	6
% pathological patients due to DM (decreased SRSL)	13	22	19	27	8	22	20
Stapedius reflex decay test							
No of patients tested				21	24	16	27
Corrected for weak or unmeasurable reflex				3	6	3	5
No of pathological patients[b]				5	7	7	5
% pathological patients due to DM				28	38	53	23

[a] 3/4 freq at one ear, 3/8 freq both ears
[b] 1 freq, 1 ear

Table VIII. *Correlations between pure tone audiometry and other audiological, ophthalmological or medical parameters*

≤49 years Insulin treated (includes 3 youngest age groups)
Sex prevalence female (54 44%)*
Proliferation of retinal vessels (18 10%)
Nephropathy (45 19%)

50–59 years Insulin and orally treated (includes 2 treatment groups)
Sex prevalence female (100 57%)
Insulin coma (67 48%)
Urinary infection (44 19%)
Nephropathy (33 23%)

60–69 years Insulin treated
Sex prevalence female (75 35%)
Acidosis (50 26%)
Cataract (50 31%)
Proliferation of retinal vessels (25 12%)
Amaurosis (25 15%)
Gangrene (37 19%)

60–69 years Orally treated
Sex prevalence female (87 42%)
Proliferation of retinal vessels (12 3%)
Amaurosis (25 8%)
Nephropathy (25 8%)

* The first figure within brackets indicates the frequency of the measured parameter for those with pathological results the other figure the frequency of this parameter for the whole age group(s)

SPEECH AUDIOMETRY

Group mean analysis of speech discrimination showed normal values (Table V) In the individual analysis each patient's discrimination score was correlated with his pure tone audiogram (Table VII) This correlation showed a fairly high incidence of patients with pathological speech audiometry, the incidence varying between 33 and 19% There also appeared to be a clear difference among the age groups in that patients up to 50 years had decreased speech discrimination more often than the patients in the four older age groups However, on an individual basis, speech discrimination scores were seldom less than 80% and most cases lie within 10% of the lower limit for normality (≥92%) (Table IX) It is mentioned that distorted speech discrimination testing was previously performed on the youngest age groups and group means were found to be normal Details regarding the results of that testing were discussed previously (Axelsson & Fagerberg, 1968)

The correlations between pathological speech discrimination and other audiological, ophthalmological or medical parameters may be seen in Table X While there was no consistent correlation between any of the tested parameters, there appears to be a correlation between poor discrimination and more severe forms of retinopathy in some of the insulin treated groups

In summary, group mean speech discrimination scores were normal While there was a fairly high incidence of individual losses, values were close to normal Considering the psychological aspects of the speech discrimination test (e g it is influenced by motivation and the general condition of the patient) as well as the stringent limits for normality (≥92%) on the test, it can be stated that speech discrimination results in our study showed little pathology There were no clear

Table IX *Speech audiometry Distribution of pathological ears*

Discrimination %	Insulin treatment Age groups					Oral treatment Age groups	
	≤29	30–39	40–49	50–59	60–69	50–59	60–69
<60			2		3		2
60–69				1			1
70–79			1		1	1	
80–84	1	1	1	2	1	1	4
85–89	2	3	2	2	1	2	1
90–92	8	11	6	4	2		1

Table X *Correlations between speech audiometry and other audiological, ophthalmological or medical parameters*

≤29 years Insulin treated
–
30–39 years Insulin treated
Sex prevalence male (90 63%)*
Duration of diabetes (22 years 15 years)
Proliferation of retinal vessels (20 14%)
40–49 years Insulin treated
Sex prevalence male (70 60%)
Proliferation of retinal vessels (25 10%)
Coronary insufficiency (37 17%)
50–59 years Insulin and orally treated
(includes 2 treatment groups)
[illegible]
60–69 years Insulin treated
Gangrene (50 19%)
60–69 years Orally treated
Aneurysm of retinal vessels (43 33%)

* See footnote Table VIII

or consistent correlations between speech discrimination and other ophthalmological or medical parameters

STAPEDIUS REFLEX TEST

Group mean stapedius reflex thresholds in dB hearing level and dB sensation level for each age group are shown in Fig 3 and Table V Mean data do not include those cases where, for whatever reason, a stapedius reflex threshold could not be obtained at the highest level tested The separation of the group mean stapedius reflexes in dB hearing level and dB sensation level increases with age and reflects the increased incidence of pure tone threshold shifts, particularly in the high frequencies and in the older patients

With the exception of the ≤29 year olds at 0 5 kHz (see Fig 3), stapedius reflex thresholds in dB hearing level lie within the normal range at all frequencies As previously stated, the audiologically uncorrected pure tone test results are within the normal range according to the criteria of Spoor (1967) Thus, although our patients had hearing within the normal limits for their age, pure tone threshold shifts in excess of 25 dB were nevertheless *present* especially in the higher frequencies and in the older patients The presence of normal stapedius reflex thresholds together with impaired pure tone thresholds in the high frequencies is pathological This is best illustrated in Fig 3 by group mean stapedius reflex sensation levels which are entirely within normal limits only for the 20–29 year old diabetics All other age groups have pathologically low stapedius reflex sensation levels at 4 kHz The 50–59 year olds also have pathologically low stapedius reflex sensation levels at 0 5 and 2 kHz when compared to Habener & Snyder's (1974) data Pathologically high stapedius reflex sensation levels were never observed in the group data

In the individual analysis each patient's stapedius reflex threshold was related to the possible existence of a conductive loss and pure tone threshold shifts (Table VII) We have divided our material into 3 groups, *namely, those in whom* stapedius reflex thresholds were normal, increased or decreased Stapedius reflex thresholds in dB

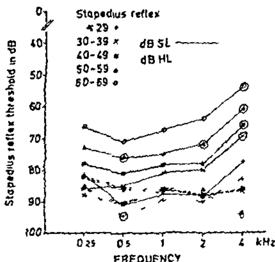


Fig 3 Hearing in diabetics Stapedius reflex thresholds for all age groups in dB hearing level and dB sensation level Encircled marks indicate statistically significant differences

Table XI *Comparison of stapedius reflex sensation level and hearing threshold level in all age groups*

Hearing threshold level	Stapedius reflex sensation level* Normal No recruitment		Increased Decruitment		Decreased Recruitment	
0 5 kHz						
Normal hearing	162		40		75	
Impaired hearing		6		0		23
1 kHz						
Normal hearing	176		29		58	
Impaired hearing		1		0		33
2 kHz						
Normal hearing	155		24		52	
Impaired hearing		13		3		45
4 kHz						
Normal hearing	68		22		50	
Impaired hearing		32		15		114
Normal hearing	62%		12%		26%	
Impaired hearing		18%		7%		75%

* Figures indicate the number of ears within each category Patients with conductive pathology have been excluded

sensation level and pure tone hearing threshold levels were then compared in these patients The results are shown in Table XI From this table it can be established that normal pure tone hearing threshold levels most often occurred in combination with normal stapedius reflex thresholds (62%—normal hearing no recruitment) Pathological hearing threshold levels most often occurred in combination with decreased stapedius reflex sen

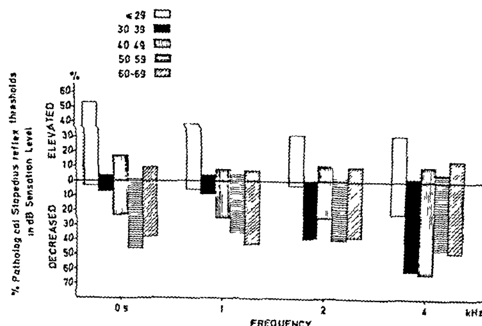


Fig 4 Hearing in diabetics Individual analysis Percent unilaterally elevated and decreased stapedius reflex sensation levels

Table XII *Correlations between increased stapedius reflex thresholds and other audiological ophthalmological or medical parameters*

≤29 years Insulin treated
DM heredity (50 32%)[a]
Nephropathy (23 15%)
Aneurysm of retinal vessels (46 35%)

30–39 years Insulin treated
Sex prevalence female (50 37%)
Hemorrhage of retinal vessels (60 48%)

40–49 years Insulin treated
Pure tone audiometry significantly better (P_{95})
at three frequencies

[illegible]

at three frequencies
Cataract (50 32%)
Nephropathy (22 10%)

60–69 years Insulin treated
Pure tone audiometry significantly better (P_{95})
at five frequencies

50–59 years Orally treated
Pure tone audiometry significantly worse (P_{95})
at three frequencies
Pure tone audiometry significantly better (P_{95})
at one frequency
Coronary insufficiency (33 12%)
Hemorrhage of retinal vessels (67 30%)
Exudate of retinal vessels (67 31%)

60–69 years Orally treated
Sex prevalence male (86 58%)
Pure tone audiometry significantly better (P_{95})
at all frequencies
Aneurysm of retinal vessels (50 33%)
Exudate of retinal vessels (33 15%)
Cataract (28 17%)

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[a] See footnote Table VIII

sation levels (75%—impaired hearing recruitment) Interestingly it was also found that 18% of the impaired hearing cases occurred with normal stapedius reflex sensation levels and thus did not show recruitment (18%—impaired hearing no recruitment) Further normal pure tone hearing thresholds often occurred together with decreased stapedius reflex sensation levels (26%—normal hearing recruitment) This means that recruitment as measured by stapedius reflex testing was present in 26% of those instances where pure tone hearing was normal Those cases with normal and decreased hearing for pure tones together with increased stapedius reflex sensation levels (12%—normal hearing decruitment, 7%—impaired hearing, decruitment) are discussed in detail later

Stapedius reflex sensation levels were most often *elevated* in the youngest age group while the frequency of *decreased* stapedius reflex sensation levels increased with age Fig 4 shows the percentages of elevated and decreased stapedius reflex sensation levels at one ear with respect to age and frequency Although *elevated* reflexes were more frequent at 0 5 kHz (53%) than from 1–4 kHz (38–31%) in the ≤29 age range, percentages of elevated reflexes were low (0–17%) and approximately equal from 0 5–4 kHz for the 30–69 year old diabetics Although 0 25 kHz is not shown (norms were not included in Habener & Snyder, 1974), forty nine patients, about equally divided among all age groups, had reflex sensation levels >95 dB at this frequency On the average, a *decreased* reflex was found in 38% of our subjects The percentage of decreased reflexes at 4 kHz was higher than at the lower frequencies for each age group

We analyzed the possible difference between the ears by noting both the dB difference between stapedius reflex thresholds and between pure tone hearing threshold levels There were 157 instances (19%) in all age groups where stapedius reflex sensation levels were >15 dB apart between ears After excluding those abnormal reflexes due to conductive pathology (54 instances) 91 of the remaining cases had ≤15 dB difference between pure tone thresholds at the ears where the stapedius reflex levels differed so much

The 78 patients with *decreased* stapedius reflex sensation levels were examined to determine whether or not their sensorineural hearing losses could be explained by noise exposure Of these 60% had some history of noise exposure but only 49% had audiograms with a configuration typically associated with noise exposure

Eight patients under the age of 30 years

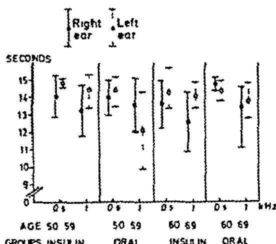


Fig 5 Hearing in diabetics Stapedius reflex decay test Group means

had a pathological *increase* in stapedius reflex sensation level Other audiological findings were either completely normal or compared well with the findings of the group as a whole except for distorted speech audiometry In these 8 patients, 5 patients (8 ears) showed pathological discrimination for distorted speech

Correlations between a pathologically *increased* stapedius reflex and audiological ophthalmological and medical parameters are shown in Table XII

While not consistent across all age groups there was a tendency for patients with a pathologically *increased* stapedius reflex to have better pure tone thresholds than the group average For older patients with oral treatment, there appears to be a high *incidence* of severe retinopathy

In summary, only for the ≤29 year olds were group mean stapedius reflex test results completely within normal limits All other age groups have pathologically *low* mean stapedius reflex sensation levels at 4 kHz This finding is largely due to a high incidence of noise induced hearing loss The 50–59 year old diabetics also were pathological at 0 5 and 2 kHz Interestingly, particularly in the younger age groups the level at which the stapedius reflex was elicited was *increased* in many cases in spite of normal pure tone thresholds Increased stapedius reflex thresholds were also found in some older orally treated diabetics with severe retinopathy

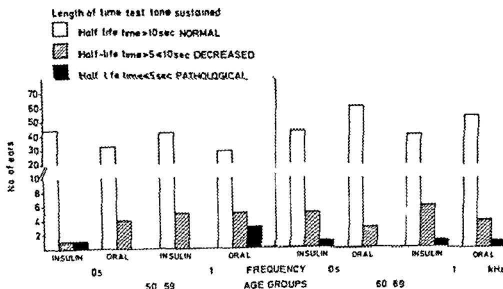


Fig 6 Hearing in diabetics Individual analysis Number of ears with pathological and non-pathological stapedius reflex decay

Table XIII *Correlations between stapedius reflex decay (half life ≤10 sec) and other audiological, ophthalmological or medical parameters*

50–59 years Insulin treated
Group mean pure tone audiometry poorer than whole age group
Aneurysm of retinal vessels (100 68%)*
Occurrence of cataract (60 32%)
50–59 years Orally treated
Group mean pure tone audiometry poorer than whole age group
Occurrence of neuropathy (30 6%)
60–69 years Insulin treated
Group mean pure tone audiometry poorer than whole age group
Amaurosis (30 15%)
Urinary infection (40 26%)
Proliferation of retinal vessels (30 12%)
60–69 years Orally treated
Group mean pure tone audiometry poorer than whole age group

* See footnote Table VIII

STAPEDIUS REFLEX DECAY TEST

Group mean results for the four older DM groups are given in Fig 5 and Table V It can be seen that the results are within normal limits (the stapedius reflex does not decay *to less than 50% of its initial value in 5 sec* or less) It can also be seen that the left ears appear to decay less than the right ears three times out of four at both 0 5 and 1 kHz Fig 6 shows the results of the individual analyses as a function of frequency and insulin or oral treatment Of the 38 individual cases (39%) of stapedius reflex half life times of less than 10 sec, decay was pathological (half-life ≤5 sec) only in 7 cases (7%) Pathological decay occurred 2 times at 0 5 kHz and 5 times at 1 kHz In one patient, the ≤5 sec half life of the response occurred at 0 5 and 1 kHz at one ear and another patient had pathologically rapid decay at 1 kHz at both ears The other 3 instances of pathological decay represent individual cases at 1 frequency Patients with a finding of stapedius reflex half-life times of ≤10 sec could be divided into three groups those with decreased, normal and increased stapedius reflex sensation levels Of the 38 instances of stapedius reflex decay (half-life ≤10 sec), 16 occurred in ears with decreased stapedius reflex sensation level, 21 in ears with normal stapedius reflexes and there was one case in which stapedius reflex decay accompanied increased stapedius reflex sensation levels Interestingly, both of the 2 instances of extremely fast stapedius reflex decay (half-life <2 sec) occurred in a patient who met our criterion for pathologically elevated stapedius reflexes bilaterally *(greater than 3 frequencies* at both ears)

Correlations for those cases with stapedius reflex decay and other audiological, ophthalmological and medical parameters are shown in Table XIII Except for the 60–69 year olds treated with insulin, patients with stapedius reflex half life times of ≤10 sec seemed to have hearing losses which exceed the group mean for their respective age groups, a result which cannot be readily explained All the 50–59 year old patients treated with insulin who had stapedius reflex decay (half life ≤10 sec) also had aneurysm of retinal vessels

In short, the group mean results of stapedius reflex decay testing are within the normal range Individual analyses showed that if the stapedius reflex half life was <10 sec, it usually ranged from 5–9 sec Test results seem to be independent of stapedius reflex results but related to poor pure tone thresholds

Discussion

Our interest in DM-related hearing testing stems from an interest in the anatomy of cochlear vessels in normal and experimental animals We have desired to correlate our experimental findings in animals with a commonly occurring clinical disease entity in man in which a general angiopathy would be expected. Diabetes mellitus fulfills these requirements Because DM often starts at an early age, it allows diabetic angiopathic changes to be distinguished from vascular changes accompanying the aging process On the basis of (1) frequent individual case reports on hearing loss in diabetics and (2) studies reporting DM-based changes in most vessels of various organs, one would also expect a similar influence on cochlear vessels with a subsequent functional degeneration It appeared that DM would be an almost ideal clinical entity for studies of microangiopathic changes This was substantiated by numerous reports of pathological findings in the auditory pathways as reviewed in the introduction However the reports on the functional influence of DM i e , audiological test results, are much less clear This may be due, in part, to methodological problems which seem to be inherent in clinical studies For example the patient's memory regarding his hearing *history, i e , type and duration of noise exposure* injuries to the head, may be unreliable Further, many of the audiological tests are highly influenced by the patient's cooperation, motivation and his physical condition Diabetics for example, are subjected to large variations in blood sugar level Patients tested after a day s work can be fairly tired Although the influence these factors have on test results cannot be exactly determined, neither can their influence be ignored

The present investigation discloses few pathological findings When the hearing in diabetics is analysed with respect to age group means the results are almost invariably normal When the diabetics were considered as individuals and each patient's audiometric findings were related to his history, the incidence of pathology is still surprisingly low Pathological findings included

1) A *pure tone loss* in the high frequencies which increased with age even after correction for ' normal ' presbycusis and history, i e , noise-induced hearing loss

2) *Speech discrimination* ability was little affected by DM

3) For the *stapedius reflex test*, many young patients had thresholds elicited at elevated sensation levels in spite of normal pure tone thresholds. On the other hand, a high number of patients also had stapedius reflex thresholds elicited at decreased sensation levels a finding consistent with the high incidence of noise-induced hearing loss in this population.

4) The *stapedius reflex decay test* showed only 7 cases in which the half-life time of the stapedius reflex response was ≤5 sec

5) The *general medical and ophthalmological examination* of these patients confirmed that they were quite severely affected by DM When attempts were made to correlate pathological audiological findings to medical and ophthalmological parameters, few correlative *findings applied to the whole* group However, there appears to be a correlation between severe retinopathy and some forms of auditory dysfunction

The present investigation compares well with previous investigations concerning high tone sensorineural loss in diabetics even after correction for age Many of the reviewed examinations found a correlation between such hearing loss and the duration of DM which we could not confirm Most investigations favour a view of a discrete premature aging of pure tone hearing in diabetics, a conclusion with which we agree

In contrast to impaired pure tone and

ometric results in the older groups, speech audiometry was most frequently pathological in younger individuals However, under the test conditions and objections previously mentioned and also considering that the pathological speech discrimination scores were almost always better than 80%, no definite statements can be made of a possible pathology in these cases

In view of our stated aim of specifically using the stapedius reflex threshold and reflex decay tests for differential diagnosis, the results are discussed in detail This aim seems *to be justified in one way* by our *finding of* pathologically increased and decreased stapedius reflex thresholds occurring quite often in conjunction with normal pure tone thresholds (39%), while the reverse, i e , normal stapedius reflex thresholds with impaired pure tone thresholds, is much less frequently the case (18%) (see Table IX) This finding suggests that stapedius reflex threshold testing may be a more sensitive indicator of hearing pathology than pure tone testing

The stapedius reflex has not been evaluated in diabetics as a group Consequently, data for comparison with the results of the present study were not available As Fig 3 illustrates, in cases with cochlear pathology, the stapedius reflex tends to occur at the same hearing level as it does in cases with normal hearing It is more informative, then, to express stapedius reflex threshold in dB sensation level By so doing, acoustic reflexes in cases with cochlear pathology would occur at reduced sensation levels, thus distinguishing them from normals However, it is also possible to obscure pathology by expressing stapedius reflex results in dB sensation level In cases where stapedius reflexes occur at normal sensation levels in spite of elevated pure tone thresholds stapedius reflex hearing levels would be pathologically elevated Jerger et al (1972) reported that in cases of N VIII pathology, stapedius reflexes occurred at normal sensation levels with elevated hearing threshold levels—thus reflexes occurred at elevated hearing levels This same finding was obtained by Popelka et al (1976) in cases with cochlear pathology resulting from acoustic trauma In our study normal stapedius reflex sensation levels together with impaired pure tone thresholds was a fairly infrequent finding (57 cases) In the patients in which it was found, 46 had either some history of noise exposure or an audiogram typical of that resulting from acoustic trauma

Two findings are apparent in the present investigation (*a*) Group mean and individual analysis showed a tendency toward *decreased stapedius reflex sensation levels which in*creased with age and frequency While this finding was most common in cases with noise-induced sensorineural hearing loss, 40% cannot be explained by a history of noise exposure or by an audiometric configuration suggestive of noise exposure, and thus, may be presumed to be DM related (*b*) Individual analysis showed a high incidence of *increased* stapedius reflex sensation levels particularly in younger diabetics and at low frequencies Increased stapedius reflex sensation levels, which were concomitant with normal pure tone thresholds, could hypothetically be explained as follows

(*a*) conductive pathology

(*b*) cochleopathy

(*c*) facial nerve or stapedius muscle pathology

(*d*) age

(*e*) normal variation

(*f*) retrocochlear pathology (acoustic nerve and/or more central acoustic nuclei or pathways)

(*a*) Middle ear pressure values exceeding ±50 mm water pressure could result in elevated or absent stapedius reflex thresholds at all frequencies (Peterson & Liden, 1972) Our patients had normal middle ear pressure values

(*b*) A pronounced cochlear microangiopathy due to DM presumably would be accompanied by hair cell damage and thus be reflected by sensorineural hearing loss and

decreased rather than increased stapedius reflexes

(*c*) A microangiopathy in the facial nerve or stapedius muscle could be reflected by an increased or absent stapedius reflex at all test frequencies. Although our material showed a frequency-specific effect, this explanation cannot be ruled out since we did not specifically test for dysfunction in the efferent portion of the reflex arc.

(*d*) The maturation of the stapedius reflex cannot be expected until the late teenage years or early adulthood (Jerger, 1970). Our pathological group of young diabetics, however, exceeded the age-based norms which reflect this maturation factor.

(*e*) Elevated crossed and uncrossed reflexes may occur as a normal variation (Jerger & Jerger, 1977). These authors consider the possibility of retrocochlear pathology only in those patients with at least one normal uncrossed reflex. In our patients, crossed stapedius reflexes were most often elevated in the low frequencies; uncrossed reflexes, however, were not tested.

(*f*) A pathology in the acoustic nerve would be expected to be reflected in directional hearing, in speech and distorted speech audiometry, in Békésy audiometry and by significant differences between pure tone or stapedius reflex thresholds between ears (Chiveralls, 1977). There was an increased frequency of pathology in distorted speech audiometry but not in ordinary speech audiometry or in directional audiometry. Only a few cases had a significant difference between pure tone thresholds between ears, but there were many cases with stapedius reflex thresholds >15 dB apart. After excluding those due to conductive pathology, 53% of the cases with discrepant stapedius reflexes occurred together with increased stapedius reflex sensation levels and 46% occurred together with decreased stapedius reflex sensation levels; normal level reflexes never occurred. The above findings speak in favor of a retrocochlear or more central localization of the disease.

Finally it should be emphasized that the finding of an increased stapedius reflex sensation level in the older diabetics in the present material could have been obscured by the high incidence of noise-induced hearing loss. This is consistent with Jerger et al. (1974) who found increased stapedius reflexes (in dB sensation level) with mild retrocochlear hearing losses, but decreased stapedius reflexes (in dB sensation level) with hearing losses exceeding 40 dB. They felt that such hearing losses probably reflected a mixed cochlear and retrocochlear involvement, and that retrocochlear findings were masked by cochlear ones. We believe this to be the case in particular in those patients with a >15 dB separation between stapedius reflex sensation levels in spite of the fact that stapedius reflexes were pathologically reduced.

Similar to stapedius reflex threshold testing, there is no information available in the literature on reflex decay in diabetics. Reflex decay testing must be considered as a more sensitive test than reflex threshold testing in that decay may be present in the absence of pathologic stapedius reflex thresholds. Of course, the absence of a measurable stapedius reflex makes the testing of decay impossible. In the present investigation, only the 50–69 year old diabetics were tested for reflex decay and in only a few cases were the results abnormal. But even with these limited results one can rank order the results in a way that indicates an increasing degree of retrocochlear pathology. This is done in the following:

Increased stapedius reflex sensation level, no pathological decay
Normal stapedius reflex sensation level, pathological decay
Decreased stapedius reflex sensation level, pathological decay
Normal stapedius reflex sensation level (with decreased hearing threshold level), pathological decay
Increased stapedius reflex sensation level, pathological decay

One patient in this study was found to have very rapid stapedius reflex decay (half life 1 6 sec) at 0 5 kHz with a normal stapedius reflex sensation level and (half life 1 sec) at 1 kHz with an elevated stapedius reflex sensation level This patient had a total of 4 pathologically elevated stapedius reflex sensation levels at both ears Although radiology in this patient was normal, such rapid reflex decay has been found to be present in patients with acoustic tumors (Anderson et al , 1969, Hall, 1977) In cases with noise induced hearing loss with normal or decreased sensation levels of the stapedius reflex, then, the rank ordering of the reflex decay test could help us to differentiate which ones had acoustic or facial nerve pathology as discussed above

We thus find two kinds of stapedius reflex pathology (*a*) decreased stapedius reflex *sensation level due, in part, to noise induced* cochlear pathology and, in part, to DM and (*b*) increased stapedius reflex sensation level, which is less easily explained since it occurred in normal hearing young patients We tend to account for the increased stapedius reflexes as a retrocochlear microangiopathy in the acoustic nerve or more central auditory nuclei or pathways There may also be additional contributions from a similar pathology in the facial nerve and stapedius muscle It could possibly represent a normal variation or result from late maturation of the stapedius reflex in young diabetics Whatever the cause of this latter finding, it should alert otolaryngologists giving occupational guidance That is the increased stapedius reflex sensation level in young diabetics can certainly indicate lack of noise protection by the acoustic reflex and cause early noise induced permanent threshold shifts in these patients

Stapedius reflex decay testing resulted in very few pathological cases Approximately 90% of our patients were normal while the remaining 10% had varying degrees of reflex decay, the half life time was pathological only in a few This low incidence of pathological findings may in part be due to our inability to employ this test when stapedius reflex thresholds are extremely elevated or absent Those cases having stapedius reflex decay are thought to have varying degrees of retrocochlear pathology

In conclusion, it must be regarded as fortunate that the hearing sense is comparatively well preserved in diabetic patients even when the disease has seriously affected the visual function In this respect, it is interesting to compare hearing with vision In the eye we have extraordinary possibilities of studying the angiopathic changes in the retina "in vivo" These changes can be graded and closely followed during the disease course Since we know the morphological correlate, we tend to pay less attention to the function of the sense organ, i e , the visual ability In contrast, we cannot study the ear "in vivo' *and assessing the organ histo pathologically* post mortem is also difficult We are thus forced to rely on tests of hearing function It is known that retinopathic changes do not impair visual function until the changes are pronounced and particularly when they affect the macula Thus it is probably incorrect for experimenters to assume that morphology is normal in ears with normal hearing function The inner ear, as most other organs, probably undergoes diabetic histo pathological changes not seen clinically

In the present investigation there appears to be a correlation between severe microangiopathic changes in the retina and different forms of hearing loss, suggesting that these cases also can be expected to have more severe morphological changes in the inner ear It appears, however, that the balance between morphological changes and functional impairment differs between hearing and vision This was confirmed in the present investigations by five patients with complete blindness due to DM who had normal hearing no such inverse relationship was ever demonstrated The explanation for this difference between the eye and ear is unknown

Summary

Diabetes mellitus is a commonly-occurring disease in which a general angiopathy is usually present. One would expect a functional degeneration of the hearing in diabetics as a result of vascular pathology. This report combines the results of two clinical studies of hearing in 205 diabetics treated either orally or with insulin who ranged in age from 16–70 years of age. The results of stapedius reflex testing as an aid to differential diagnosis is stressed. When the hearing in diabetics is analyzed with respect to age group means the results are essentially normal. The individual analyses showed a few abnormal findings but the incidence of pathology was still low. Pathological findings included high frequency pure tone hearing loss, slight decrease in speech discrimination ability, some abnormally elevated and some abnormally decreased stapedius reflex thresholds and a few cases of abnormal stapedius reflex decay. The general medical and opthalmological examination of these patients confirmed that they were quite severely affected by diabetes mellitus. The results suggest a premature aging of hearing for pure tones in diabetics. There appears to be a correlation between severe microangiopathic changes in the retina and different forms of hearing loss. The significance of the results is discussed.

Zusammenfassung

Diabetes mellitus wird sehr oft durch eine Angiopathie kompliziert. Wie in anderen Organen konnte man bei Diabetikern eine funktionelle Degeneration des Gehörs als Resultat der Angiopathie erwarten. Diese Untersuchung berichtet uber das Gehor bei 205 Diabetikern die entweder peroral oder mit Insulin behandelt wurden und die 16–70 Jahre alt waren. Die Messung des Stapediusreflexes als ein Hilfsmittel der Differentialdiagnose wird betont. Das Gehor der verschiedenen Altersgruppen der Diabetiker zeigte meistens normale Befunde. In der individuellen Analyse konnte man einige abnorme Befunde machen. Die Frekvenz von patologischen Befunden war je doch niedrich und bestand aus einer Schwellenabwanderung der hohen Frekvenzen bei Reintonaudiometrie, einer leichten Verminderung des Sprachverstehenvermogens, einiger abnorm erhöhten und einiger abnorm verminderten Schwellen des Stapediusreflexes und selten einer abnormer Ermudung des Stapediusreflexes. Die allgemeine internmedizinische und [illegible]logische Untersuchung dieser Pat[illegible] das sie ernst von der Krankhe[illegible] waren. Die Befunde deuten auf [illegible] fruhten Alterungsprozess [illegible] Diabetikern. Es scheint [illegible] zwischen schwere mikro[illegible] Veranderungen der Retina [illegible] verschiedenen Formen von [illegible] andersets vorzuliegen [illegible] verschiedenen Befunde [illegible] kutiert

Acknowledgements

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OTO-LARYNGOLOGICA
SUPPLEMENT 357

A New Hypothesis on the Plurifactorial Etiology of Ménière's Disease

BY
MICHELE ARSLAN

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ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 357

A New Hypothesis on the Plurifactorial Etiology of Meniere's Disease

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Abstract

Meniere's disease is characterized by an extremely long trend this fact, as in other diseases of very wide chronicity, permits one to formulate a special type of hypothesis regarding the aetiology and the pathogenesis of the disease, it is comprised of many individual factors, of differing chronology and which are connected to each other with successive causality This hypothesis was founded on a large body of data concerning the multiple histopathological effects on the inner ear of an initially inflammatory disease of the middle ear

Introduction

The identification of the primitive etiology of diseases having a long chronicity, and which have evolved over a number of years, is a difficult and at times impossible task (except, of course, in the case of diseases with an inflammatory, tumoral or traumatic origin, etc) The analysis of the formal aspects of chronic diseases of this type, and of variations throughout their long duration, can indeed bring together a series of significant individual etiological co-factors, thus allowing the demonstration of a causal relationship between certain co-factors having different chronologies, but it rarely reveals the true nature of the entire etiological force which supports the disease over a long period of time this may be so well hidden as to prevent the forming of a single etiological hypothesis The scientist is frequently obliged to resort to the identification of a sequence of many different etiological factors, some of them unknown, which succeed one another and exercise their harmful effects at different times it may frequently appear impossible to qualify the actual mechanisms of this chain of factors

Meniere's disease (M 's d) or *endolymphatic hydrops*, is a typical example of the forms described above (in internal medicine there is no shortage of examples of diseases of this type e g diabetes, hepatic cirrhosis, etc , in ophthalmology, glaucoma is another example)

It is a well known fact that the clinical onset of M 's d often becomes manifest in a period of otherwise excellent health, with a sudden attack of vertigo, symptoms of hypervagotonia (vomiting or nausea, cold sweats, etc), and severe postural disturbances after a few days this clinical picture resolves and the patient can again enjoy his previous good health, perhaps for a long time, but the disease has already been with him for years, even decades, revealing itself in only one subjective symptom which often goes unnoticed by the patient, viz *slight unilateral deafness*



Fig. 1. Extensive fibrosis of the tunica propria of the pars rugosa (From Altmann & Zechner, 1968)

I. The First Group of Etiological Factors in Meniere's Disease

Histological findings

For several decades it has been impossible to explain the etiological mechanisms of M.'s d. One of the questions asked by all otologists and which has actually given rise to four international symposia on M.'s d. in the space of four years (Chicago 1973, Los Angeles 1974, Padua 1974, Friburg 1976), is the following: what is the etiology of the endolymphatic hydrops? Is it an etiology consisting of one single or several factors? In many chronic diseases (non-inflammatory, non-traumatic, and non-neoplastic) of various organs or somatic systems, and in chronic unilateral diseases affecting twin organs (kidneys, lungs, cerebral hemispheres, etc), the mechanisms of etiology and pathogenesis have been identified, almost always by the contribution of the data obtained from pathologic anatomy These data also exist for M.'s d , but they have only been partly evaluated. Great importance has been attached to the hydropic lesions of the endolymphatic canal; there is *in fact* a very rich literature on these lesions and many pathogenic hypotheses have been proposed (rupture of the wall, according to Schuknecht and his school; "spastic-atonic arteriolar-capillary state", according to Williams; "disproteinemia and depolymerizing action of hyaluronidase", according to Godlowski, (1972); primitive "troubles of secretion and/or absorption of endolymph", etc.). But histopathological studies on the entire inner ear, carried out during autopsies on patients who died from various causes and who, during their lifetime, had been affected by M.'s d. (which is never fatal), and especially histological examinations of the petrous bones collected in the "temporal bone banks" set up in many Institutes of Otology (Vienna, Boston, Los Angeles, Chicago, etc), have demonstrated not only the constant presence of the hydropic lesion (or of collapse) of the endolymphatic duct, but in *all* cases, without exception, of subjects who had suffered from M 's d. in life, constant and frequently severe alterations of the bony structures and of the endosteum of many canalicular formations of the inner ear (semicir-



Fig 2 Newly formed bone in the lumen of perilymphatic space of the superior semi circular canal (From Altmann & Zechner 1968)

cular canals, vestibule, endolymphatic duct) The histopathological findings may be divided roughly as follows)

1) a distension (i e the hydrops), or a collapse of the endolymphatic duct,
2) pathological fibrosis, of a distinctly post-inflammatory type, of the walls of the membranous labyrinth,
3) lesions of the *bony walls* of the labyrinthine cavities, consisting of fibrosis and stenosis-producing calcifications of the supporting mesenchyma of the membranous labyrinth, and of the endosteum These lesions have the histopathologic appearance of the after effects of inflammations which have evolved slowly over a number of years, of fibrous inflammatory processes, with perivasculitis, absence of hyaluronic acid, and laminar ossifications which at times block the bony walls containing the various labyrinthine structures, obstructing preferentially the endolymphatic duct, etc (Altman & Zechner, 1968, Zechner, 1974) These lesions may be bilateral, but in almost all cases profound differences may be observed between the extent of the lesions on either side (Figs 1, 2, 3 ")
4) inhibition of the pneumatization of the temporal bone and, in particular, of its petrous part Kaufmann Aremberg Rask-Andersen, Wilbrand & Stahle, 1977, Arslan, 1972, which will be discussed below

The question that immediately comes to mind when faced with such *constant* histopathological objectivity is this what could be the causal factors of histopathological pictures of this nature?

Inflammations of middle ear in infancy and the possibility that they may be the primary etiological factor of M.'s d

Since the time of Wittmaack, it has been known that acute otitis media in infancy always determines an inhibition of the pneumatization of the mastoid, with varying degrees of extension and localization distributed through out the various phases of the disease It is also known that the clinical pictures of inflammation of the middle ear in the first and second stages of infancy differ quite markedly 1) they are frequently difficult to identify because the patient is almost always too young to be able

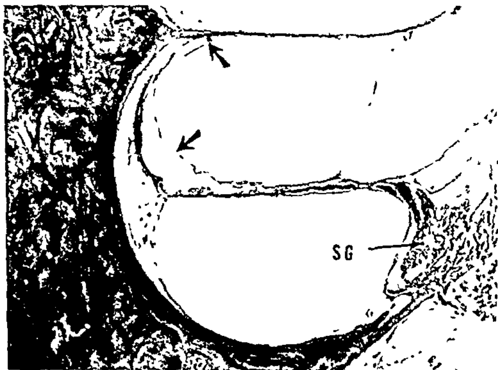


Fig 3 Collapse of the endolymphatic system (From *Schuknecht, 1968)* Collapse of the end canal can be *considered as an alternative lesion of the hydrops* hy pothetically these two lesions could correspond to the opposite nystagmus (irritative ', i e collapse paralyt ic , i e hydrops) As a matter of fact, in every Me niere s attack, irritative nystagmus appears primarily and is followed by a paralytic nystagmus

the ear, 2) in the great majority of cases y are unilateral, short-lived, and respond in few days to antibiotic treatment, 3) the "reactivity ' of the mucosa of the middle ear and of the antromastoid cells is very limited at that early age, due both to the lack of an immuno-defensive mechanism and to the fragility of the structures of tissues whose morphogenesis is as yet incomplete

Possibility that, in some cases of acute otitis media in infancy, there may be an inflammatory labyrinth reaction due to transudation of secretion products through the round window membrane

This highly important fact is as follows in flammation of the middle ear and of the Eu stachian tube itself (which is nearly always inflamed too), can in some cases exercise a harmful effect through the round window (of which Arnold (1977) and Arnold & Ilberg (1972) have demonstrated the "physiological' permeability) Paparella & Brady (1970) recently showed how fleeting otitis media without any functional labyrinthine sign sometimes determines reactions in the labyrinthine tissues close to the medial face of the round window that is, its membrane can be transuded by "toxins", or by substances with antigenic properties, or by "noxae" in a braoder sense, produced by the inflammation of the middle ear (Fig 5)

We consider as well-founded the hypothesis, already put forward by Tumarkin (1966), according to which these inflammatory substances, reaching the endolabyrinthine structures through the round window (structures which, unlike the mucosae of the middle ear, are sterile and have no immuno-defensive mechanisms), provoke there a slowly developing mesenchymal reactivity, which is expressed in those constant histopathological alterations referred to above

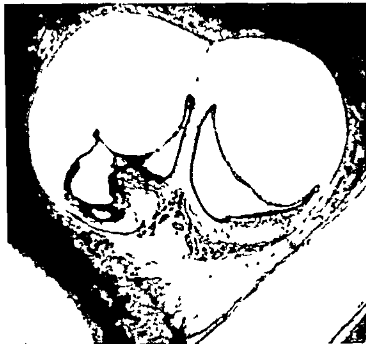


Fig 4 Experimental Meniere's disease collapse of endolymphatic canals (From Arslan 1972)

As a matter of fact, in these subjects the round window membrane is transuded by "microtoxins" provoked by the preceding inflammation, these "microtoxins" arrive in the perilymphatic spaces and even cross Reissner's membrane, causing a fragility of the very fine vascular and cellular structures charged with the secretion and absorption of the endolymph and the perilymph (stria vascularis, perilymphatic vessels, spiral ligament, endolymphatic duct, etc)

Significance of the prevalent unilaterality of M.'s d.

A valuable confirmation of the primary etiological factor can be obtained also from the statistical study of the uni- or bilateral incidence of M 's d All authors agree that, in the great majority of cases, M 's d occurs most frequently with clinical evidence on only one side the figures point to values between 85% and 92% (e g statistics of Hallpike & Cairns, 1938, Naftalin, 1958, Harrison, 1958, Hallberg, 1968, Le Roy E Edgecock, 1968) This statement is of definite value in the discussion on etiology It is not rendered invalid by the fact that, in its advanced phases, the disease may also become manifest on the unaffected side (in this respect, see the recent paper by Greven & Oosterveld (1975) at this point we only wish to emphasize that there is nearly always in the early period of the disease, a considerable chronological prevalence of unilateral lesions (Figs 6, 7)

When considering this problem in the pathology of a *twin* organ (kidney, lung, ear, etc), i e the problem of the localization of a disease, no one can deny the validity of the following two considerations (A) If, as in the great majority of cases, the disease strikes only one side, a local pathogenic mechanism must be at work as the "first pathogenic mechanism" (B) In unilateral disease a local factor is obviously *considerably more important* than the secondary general factors, which we will discuss later

In fact, if we consider how many authors state that the etiology of M 's d is linked *only* with disturbances of the osmolarity, of the hydric or glycid exchange, and of the metabolism in general, the fact that M 's d is



Fig 5 Experimental demonstration of the permeability of the round window membrane with thorium-dioxide (electron microscopy) (From Arnold & von Ilberg) *Th* Thorotrast, *L* middle ear, *Mv* Micro villi *ZO* Zona occludens, *Mpv* vesicle including thorium oxide, which has crossed the round window membrane, *E* epithelial cell *Bm* basal membrane, *Ezr* extracellular space *F* nucleus of a fibrocyte *M* mitochondrium, *F* collagen fibre, *ds* compact vesicle

largely unilateral in nature lends value to another aspect of the primary local etiological factor, namely the "locus minoris resistentiae", it is to this factor that we attribute the constant histopathological picture of the inner ear described above

At this point it is opportune to make a consideration of a general nature with regard to the etiopathology of twin somatic organs (kidneys, lungs, cerebral hemispheres, etc) if one of the two organs is affected by disease, or of both are struck with different intensity, the more affected organ possesses a "predisposing factor' which, in extreme cases, may have given no clinical sign of its presence and may prove almost impossible to identify semeiologically In histochemical examinations of the inner ear of patients who had suffered from unilateral M s d , Godlowski too found protein alterations of the membranous structures (absence of hyaluronic acid, mucopolysaccharide alterations, etc), he admits the necessity of a local "contributing factor" which has allowed a greater vulnerability to lesion of the affected labyrinth

On the other hand, if we then consider the primary (monofactor etiology), localization of *generalized* disease processes in twin organs of our organism, we find a fact that fully confirms the validity of our previous reasoning, in the great majority of cases, the two organs, the right and the left, are affected with the same intensity to remain within the sphere of otorhinolaryngology, when patients are suffering from peripheral arteriopathy, from more or less severe diabetes, from ionic deficiency, from more or less severe disturbances of the multiple hepatic functions, from lack of hor-

Table I *Patients Studied*

	NUMBER
Total patients	205
Male	114 (56%)
Female	91 (44%)
Patients with Meniere's disease	
Unilateral	187
Bilateral	18 (9%)
Ears	
Total	223
Right	101
Left	122
Age years	
Range	22 79
Mean	53

Fig 6 Distribution of unilateral and bilateral Meniere's disease (From LeRoy D Hedgecock 1968)

monal balance, etc, we confirm the bilaterality of the lesion (for example deafness from arteriosclerosis from senescence, from inflammatory processes of the upper airways) The same fact occurs in ophthalmology for example, diabetic retinitis, retineal angiosclerosis, etc

Inhibition of the pneumatization of the temporal bone as a consequence of otitic and/or tubaric inflammation (Wittmaack's theory)

Since we began studying the pathology of M's d, we have observed during operations with ultrasound or with osmotic induction, that many Meniere patients present signs of late consequences of old inflammations of the middle ear and of the antrum These signs could sometimes be seen as a small line of scarring at the level of the promontory, sometimes in the mucosa lining the bony walls of the middle ear, with connective aspects typically classifiable as small scars caused by precedent old inflammations

In the majority of these cases, the proof that the subjects had had otitis media even 30 or 40 years previously (in their infancy the disease often passed unnoticed), could be established only by means of systematic radiography of the mastoids

According to the theory formulated by Wittmaack (1926), (who was one of the greatest researchers in the pathological anatomy of the ear), otitis media in infancy determines a partial or total inhibition of the physiological process of pneumatization of the mastoid in fact, if we make a radiograph of the mastoids in otopathic adult subjects, even though they present no tympanic scarring or hypoacusia, it is *frequently possible to find partial or total* eburnation of the mastoids or an abnormal *pneumatization of the temporal bone in all* cases this judgement must be based on comparison with the pneumatization of the healthy side this often constitutes proof that the patient has had acute otitis media in the past (Figs 8, 9, 10, 11)

CALORIC TEST RESULTS

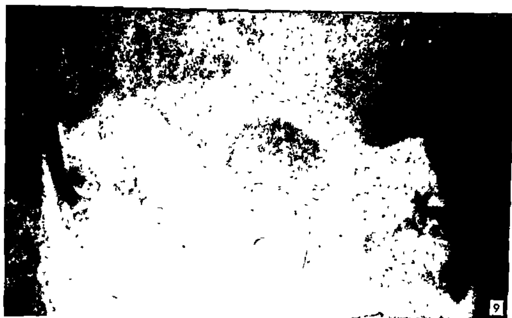
	Hallpike's (1942) Series (%)	*Cawthorne's (1954) Series (%)*	*DeKleyn (1939) Series (%)*	*M S H (%)*
Unilateral abnormalities	86	91		88
Canal paresis	49	71	73	55
Directional preponderance	21	8	11	25
Combined lesions	16	12		8
Bilateral abnormalities	6			10

Fig 7 Distribution of unilateral and bilateral Meniere's disease









Figs. 8, 9, 10, 11. Some examples of unilateral inhibition (either partial or extensive) of the mastoid pneumatization


10


10


11


11



Fig 12 The inhibition of the pneumatization by Meniere patients *A*=Petrous pyramid of a patient with Meniere s disease Narrowness and anatomical dislocation of the external opening of the vestibular acquaeduct (white arrow) *S*=sinus lateralis Failure of pneumatization (black arrow) *B*=Petrous pyramid of a normal subject Large opening of the vestib acquaeductus (white arrow) Pneumatization with large cells (black arrow) *S*=S nus (From Stahle & Wilbrand 1974)

Table I *Tomographic classification of the type of periaqueductal pneumatization pattern of the petrous pyramid and the length of the VA in a normal population compared with patients with Meniere's disease*

	Type 1 Large cell pneumatization	Type 2 Small cell pneumatization	Type 3 No pneumatization
Normal subjects (*n*=32 ears)	*n*=11	*n*=14	*n*=7
Length of VA mm	10 3	8 2	7 4
(Range mm)	(7 2–13 5)	(6 1–9 9)	(6 7–8 1)
%	34	44	22
Meniere disease (*n*=86 ears)	*n*=0	*n*=28	*n*=58
Length of VA mm	None	7 7	6 9
(Range mm)		(5 9–11 7)	(4 3–10 2)
%	0	26	74

"Comparison of normal population to patients with Meniere disease (From Kaufmann Arenberg Rask Andersen Wilbrand & Stahle 1977)

Recent research by Hermann & Kramer (1973) on 244 children, has confirmed Wittmaack's theory attacks of seromucous otitis in infancy cause inhibition of the pneumatization of the temporal bone

Recent research on the pneumatization of the temporal bone in Meniere's disease

Stahle & Wilbrand (1974) made a systematic study of the radiological appearance of the vestibular acqueduct in Meniere patients (Fig 12) In the majority of subjects they found (*a*) shortening of the acqueduct, (*b*) lack of pneumatization of the petrous bone such as is observed in normal subjects We have already pointed out how previous research by Wittmaack had led him to state that the morphology of the endolymphatic sac varies according to the degree of pneumatization of the pyramid In 28 out of 35 Meniere patients, Clemis & Valvasson (1968), found that the tomographic examination of the temporal bone was incapable of visualizing the vestibular acqueduct Judging by these results, House (1976), Fisch (1976) indicated that this fact *must come under suspicion for the difficulty* experienced in finding the endolymphatic sac in decompressive' surgery in M 's d It also appears that, for this same problem, particular importance must be attached to research recently carried out by the Uppsala school (Stahle, Kaufmann Aremberg, Rask Andersen & Wilbrand, 1977), this team carried out precise anatomical, tomographic and statistical research on the frequently profound variations in the endolymphatic sac and the vestibular acqueduct, and on the pneumatization of the petrous bone, in a large number of Meniere patients These studies, which were carried out with the utmost precision following parameters of exact comparison with normal subjects, have shown, among other things, that in 74% of Meniere cases there is no pneumatization of the petrous bone, this incidence falls to 22% in normal subjects (Table I and Fig 13) These results give full confirmation to the data gathered by M Arslan and colleagues (1972) (Figs 8, 9, 10, 11)

Arslan et al (1972), had in fact carried out systematic research on the pneumatization of the mastoidean and periantral portion of the temporal bone in over 400 subjects suffering from M 's d in the great majority of cases, alterations were found in the pneumatization of the mastoid and of nearby areas these alterations go through a fairly wide range of variations, and are composed of more or less widespread areas of eburnation, of inhibited pneumatization, or of dimensional hypoevolutism of the mastoid, etc The significance of the structural abnormality, in the sense indicated



Fig 12 The inhibition of the pneumatization by Meniere patients A=Petrous pyramid of a patient with Meniere s disease Narrowness and anatomical dislocation of the external opening of the vest bular acquaeduct (white arrow) S=sinus lateralis Failure of pneumatization (black arrow) B=Petrous pyramid of a normal subject Large opening of the vestib acquaeductus (white arrow) Pneumatization with large cells (black arrow) S=Sinus (From Stahle & Wilbrand 1974)

above, is corroborated by comparison with the healthy side These alterations are sometimes bilateral but the great majority are *always* on the same side as the diseased ear Our data are therefore in perfect agreement with the results of the research quoted above, on the faulty pneumatization of the petrous bone in Meniere patients

II. The Second Etiological Factor in Meniere's Disease

Before dealing with the problem of the second specific etiological factor in the Meniere attack, the typical clinical picture of the disease must be synthesized This undertaking may seem trivial and unjustified, since if it is true that today the consensus of the otological literature states that there are a number of syndromes similar in expression or form to that of Meniere, an attempt to describe a typical clinical picture could appear pleonastic, or even lacking in nosological reality

In fact, the basic symptoms of the disease, namely perceptive deafness and its fluctuations, vertiginous attacks, tinnitus and pathological hypervagotonic phenomena (vomiting, nausea, cold sweats, which always accompany vertigo, etc), are not very often simultaneous, nor of equal intensity in other words there is neither symptomatological nor chronological coordination of these symptoms Apart from hypoacusia and tinnitus which are the first to appear (frequently unnoticed by the patient), and which are usually fluctuating in nature, the other symptoms are episodic phenomena, separated by intervals of otherwise excellent health In the great majority of cases, the first symptom noticed by the patient is the vertiginous attack, in itself this symptom is non specific, as it may derive from alterations, even of a transitory nature, of the complex vestibular system (very varied from the anatomical point of view with respect to topographical levels, including the vestibular receptors, the nervous trunk, the many nuclei, the complex central vestibular connections) The authenticity of the diagnosis of M 's d must therefore be confirmed by precise and exhaustive functional tests

It therefore appeared logical to attribute the pathogenic mechanism of the attack to metabolic disturbances (alterations of osmolarity, of alkalosis, of hydric and/or glycid exchange, of renal function, of protein exchange, etc) the Rome school (De Vincentiis et al , 1970, 1972), (Stahle, 1972, etc), offered the first valid demonstration of this attribute There are in fact close links between the general mechanisms of the peripheral circulation, the osmolarity, the potassium and sodium exchanges etc , and the production and absorption of the labyrinthine liquids, especially endolymph

Thus it is evident that the vertiginous attacks, which represent the hinge syndrome of M 's d , have no direct etiological connection with the first group of etiopathigenic factors, i e , with the histopathological lesions of the temporal bone (which are of a *permanent* nature) nor with the hydrops of the endolymphatic system These are permanent lesions which, if they were the *direct* cause of the vertiginous attacks (in so far as in the pathogenesis of every single factor disease, there is frequently a relationship between the formal lesion and the consequent pathofunctional phenomenon), would be such as to produce *constant* vertiginous disturbances in the Meniere patient

The exact opposite occurs in the cochlear receptor, where the hydrops of the cochlear endolymphatic duct determines a permanent compression of the *membrana tectoria* on the

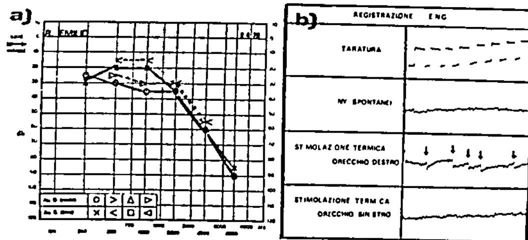


Fig 14 Audiogram and electronystagmogram registered by a patient suffering from severe nephrosis with osmolarity troubles of high degree In this patient, vertigo has been observed, but *never* a typical Meniere s attack (From Arslan, Mozzo Calvelli 1976)

organ of Corti in this case we can easily see its direct consequence in the hypoacusia which, though characterized by fluctuations, represents a *permanent* symptom in sufferers from M 's d

It can therefore be deduced that, when the endolymphatic hydrops does not determine the vertiginous attack, two conditions must be fulfilled (1) normal cupolar mobility, guided by its physiological parameters (movements during angular acceleration, arising from rotations of the head), (2) an otolithic function which is physiologically active during angular accelerations, rectilinear accelerations and oblique movements of the head Consequently these physiological mechanisms may be exercised even when there is a permanent endolymphatic hydrops

We must therefore consider that only the biochemical or hydrodynamic variations of the endolymph, due to sudden plasmatic variations, to variations of the potassium content, or to circulatory variations, by altering the biochemical and biopyysical values of the endolymph, cause a pathological modification of the cupolar mobility and of the shearing forces acting on the otoliths, in this way there occurs a distortion of the messages directed towards the vestibular nuclei with a consequent rupture of the physiological nuclear "fusion" between the excitations on one side and those on the other

Labyrinthine alterations in severe nephropathies

In order to elucidate the plurifactorial pathogenesis illustrated above, researches were carried out on the labyrinthine alterations in subjects with chronic nephritis, undergoing dialysis or with an artificial kidney (Mozzo & Calvelli, Padua, 1977) In these subjects we did not observe sudden severe vertiginous attacks, with the specific appearance of those seen in M 's d , nor neurovegetative phenomena, such as sweating, vomiting, nausea, pallor, etc instead, they presented mainly a state of light vertigo and insecurity of balance, especially when walking Although this disturbance sometimes lasted quite a long time (even over a number of days), there were periods of almost complete remission In most cases the appearance of vertigo followed that of the simultaneous lesion of the hearing, even several months later *However, we never observed an intense symptomatology, with fits of the type found in the Meniere attack*

In some of these severely dysmetabolic and

dysosmolaric subjects whom we had under observation, a decline, at times quite marked, of the vestibular reflectivity was noted, *though this was always bilateral* with *symmetrically* quantitative and nystagmographic values (Fig 14)

On the basis of these findings it can reasonably be supposed that both the state of subvertigo and the progressive decline of the peripheral vestibular reflectivity in patients suffering from renal function and metabolic disturbances of varied origin, are determined by the progressive alterations of the physicochemical constants which occur in these subjects, in whose humoral liquids—and consequently also *in the endolymph and the perilymph*—there accumulate metabolic substances which are not sufficiently eliminated on account of the current nephrosis

Also in patients undergoing dialysis or with an artificial kidney, the vestibular apparatus presented, though to a lesser degree, a diminished vestibular reflectivity equal to less than half the values found in normal subjects (Mozzo & Calvelli, 1976)

However, from a subjective point of view, the somatic balance and posture in all these cases were only slightly affected, although there appeared almost constant vertiginous attacks of moderate degree, always short-lasting, which occurred during sudden movements *of the head*

Conclusions (Table II)

All we have stated above leads us to believe in the possible existence of a plurality of the etiological and pathogenetic factors of M 's d *These factors are of course interrelated* One factor is that which causes a definitive severe anatomical alteration of the endolymphatic duct and connective structures both cochlear and vestibular (the constant presence of hypoacusia is the first pathological consequence of this alteration), while allowing cupolar mobility and otolithic function to proceed according to normal parameters, though *restricted within very narrow critical limits* Another factor is the sudden dysmetabolic trouble which provokes the typical Meniere attack *because the anatomical situation of the vestibular structures has been altered*, especially on one of the two sides In the great majority of cases, this is the side which shows all the typical symptoms of Meniere's disease

If the primary pathogenic factor of M 's d were, as so many authors affirm, osmolarity troubles, metabolic disturbances, K and/or Na deficiency, hypothyroidism, allergy, hypoglycemia, psychoneurosis, "*infection of the upper respiratory tracts, or of the sex organs*" (see table III of Godlowski, 1972), we wonder why there are thousands and thousands of patients who suffer from these general diseases and yet are not affected by Meniere's disease If we were to calculate by computer the incidence of Meniere's disease in these tens of

Table II

Plurifactors

1) Otitis media in childhood
2) In some cases of these patients middle ear secretion products have transuded the round window membrane (Paparella Arnold von Ilberg etc)
3) Inhibition of the pneumatizatoin of the temporal bone and of the bone and mesenchymal walls of the uppe[illegible]

[illegible]

5) Prevalence in the first period of M s d of unilateral lesions

Monofactor

6) Fleeting osmolarity troubles which decompensate the endolymphatic circulation (Celestino Stahle etc) *already functioning with critical possibilities*

Acta

OTO-LARYNGOLOGICA

SUPPLEMENT 358

Acoustic Trauma in Cats

Cochlear Pathology and Audiotory-nerve Activity

BY

M. CHARLES LIBERMAN and NELSON Y.S. KIANG

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ACTA OTO-LARYNGOLOGICA

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Acoustic Trauma in Cats

Cochlear Pathology and Auditory-nerve Activity[1]

BY

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BOSTON 1978

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Introduction

It has long been known that exposure to intense acoustic stimuli can cause permanent damage to the tissues of the inner ear. (See Kellerhals (1972) for a review of some of the earliest acoustic trauma research.) In early animal experimentation, it was shown that the locus of the noise induced damage within the cochlea could be varied by changing the spectral content of the traumatic stimulus (Stevens, Davis & Lurie, 1935, Lurie, Davis & Hawkins, 1944, Smith, 1947). As the frequency of the stimulus was increased, the damage occurred progressively nearer the base of the cochlear partition. These data were used in early attempts to reconstruct the relationship between distance along the cochlea and the frequency of maximal stimulation (Stevens, Davis & Lurie, 1935).

In the present study, acoustic trauma was used as a method of creating varied patterns of cochlear lesions. The structural changes, evaluated with light microscopy, were compared to the functional changes seen in the activity of single, auditory nerve fibers. Since most auditory-nerve fibers innervate only one sensory cell (Spoendlin, 1971a and 1973), recordings from one of these fibers can be used as a monitor of the functional state of a restricted cochlear region. The functional state of the entire cochlea can be assessed by sampling activity of single fibers throughout the nerve bundle. The correlation of the structural changes in the cochlea with abnormalities in auditory nerve response can provide information on the normal role of cochlear structures in determining auditory nerve activity.

The data on abnormal auditory-nerve activity can also be important to an understanding of the complex changes in hearing following acoustic trauma. Since the array of auditory-nerve fibers is the only channel for auditory information reaching the central nervous system, an understanding of the noise induced alterations in single unit discharge patterns is key to understanding the physiological mechanisms underlying these behavioral changes.

Methods

Healthy cats with clean external ears were chosen for this study. The electrical recordings from the auditory nerve were made using methods similar to those described by Kiang et al (1965) Several weeks before the recordings, some of the cats were exposed to high-intensity noise Other animals did not undergo the noise exposure and were used as control animals Four of the control animals were born and raised in a soundproofed chamber (Liberman, 1978) After the single-unit recordings, the temporal bones of all tested ears were prepared for histologic examination with the light microscope

I NOISE EXPOSURES

[illegible] effort was made to use young animals for the experimentally noise-exposed [illegible] in order to minimize possible preexisting ear pathology Most of the cats weighed less than 1 5 kg at the time of the noise exposure (Table I) In order to have better control of the noise exposure, the cats were anesthetized with Nembutal (80 mg/kg body weight) and were positioned in a headholder so that the left tragus was 1–2 cm from the mouth of an acoustic driver (Atlas PD-60) The generation of the traumatic stimuli and typical acoustic spectra have been described elsewhere (Kiang, Liberman & Levine, 1976) The sound pressure at the tragus was monitored throughout the exposure by means of a 1/4-inch condenser microphone (Bruel and Kjaer) During one typical exposure (to a noise band centered at 6 kHz), the sound pressure at the entrance to the (nominally unexposed) right ear was also measured and found to be 40 dB lower than on the left side Each animal was exposed for one session, usually lasting two hours The spectrum and level of the noise differed from animal to animal (Table I) The exposure parameters were varied in order to obtain different patterns of cochlear damage After exposure, the animals were allowed to survive for two to forty weeks before recordings were made from the auditory nerve Most of the animals had survival times of four to eight weeks By this time, most of the temporary components of noise-induced threshold shift are reported to have disappeared in behavioral experiments (Miller, Watson & Covell, 1963)

II RECORDING FROM SINGLE AUDITORY-NERVE FIBERS

On the day of the recordings, the animal was anesthetized by an intraperitoneal injection of Dial (75 mg/kg body weight) The surgical exposure has been described elsewhere (Kiang et al , 1965) In order to exploit fully the tonotopic organization of the auditory nerve (Sando, 1964, Kiang et al , 1965), an attempt was made to maintain the electrode penetrations at a constant angle with respect to the cat's head, from penetration to penetration and from animal to animal After retracting the cerebellum, the surface of the auditory nerve was visualized with an operating microscope, and the recording microelectrode was oriented 45° from the horizontal plane and 10–15° from the sagittal plane The entry point of each microelectrode pass through the nerve was noted on

Table I *Information concerning noise-exposed cats*

Weight (column 2) refers to body weight at the time of the noise exposure Columns 3–6 give the parameters of the exposure stimulus in each animal Stimulus level refers to the root mean square (rms) sound pressure level (SPL) measured at the entrance to the ear canal Survival time (column 7) is the number of days between noise exposure and physiological recordings

Cat no	Weight (kg)	Center frequency (Hz)	Bandwidth (cycles)	Stimulus level (dB SPL)	Duration (hours)	Survival time (days)
78	1 24	6 000	200	107	2	305
77	2 04	3 000	50	110	2	58
76	2 55	12 000	400	118	2	57
74	0 68	1 500	50	110	2	37
73	1 84	6 000	200	116	2	30
72	1 70		White noise	110	4	43
71	1 30	6 000	100	110	2	273
70	0 76	1 500	50	108	2	54
68	1 02	12 000	400	117	2	21
67	0 91		White noise	106	4	27
66	0 79	3 000	50	108	2	57
65	2 29	12 000	250	110	2	64
64	0 79	1 500	50	110	2	28
63	0 91	1 500	50	108	2	21
62	1 25	3 000	50	110	2	15
61	1 98		Low pass	115	4	49
60	2 27	1 000	25	105	2	34
59	1 98	3 000	30	105	2	27
58	2 30		Low pass	120	2	28
57	1 81	3 000	25	100	2	54
55	2 07	3 000	25	105	1	34
50	1 67	5 000	50	102	2	55
48	1 22	6 000	100	102	2	54
45	1 02	6 000	111	100	1	47
43	0 79	6 000	50	105	2	24
36	1 62	6 000	200	105	2	37

a calibrated drawing As the electrode was advanced by means of a hydraulic manipulator, the depth in the pass at which each unit was encountered was recorded This procedure was followed in experiments on unexposed as well as exposed animals

To compare data among animals, it was necessary to establish reproducible landmarks on the nerve bundle by which the calibrated drawings could be correctly superimposed The landmarks chosen were the posterior boundary of the inferior division of the vestibular nerve and the posterior boundary of the auditory nerve Both were usually easy to locate Since the inferior branch of the vestibular nerve sweeps diagonally across the course of the auditory nerve, the posterior borders of these two structures serve to align the calibrated drawings in both the anterior posterior and lateral medial dimensions (Fig 4) The regularity of the tonotopy permitted more confidence in the completeness of unit sampling and reduced the amount of time required to obtain complete samples

In some of the experiments, electric shocks were used to stimulate single units These shocks served to locate units with low rates of spontaneous discharge and high thresholds for acoustic stimuli In the earlier experiments (MCL 36-MCL 45), this electric stimulation was accomplished by passing 5-msec current pulses of 100–300 microamperes, at a rate of 10/second, between a wick electrode on the round window membrane and a wire electrode on the bony capsule over the apex of the cochlea In the later experiments, similar electric shocks were delivered directly through the recording microelectrode

The means of presenting acoustic stimuli and the techniques for recording and process-

ing single unit activity were essentially as de scribed previously (Kiang et al 1965) Thresh old tuning curves were obtained for each unit by means of an automated procedure (Kiang Moxon & Levine 1970 Liberman 1978) The tuning curves presented here correspond to those frequency-intensity combinations which in a given unit elicit an increase in the rate of discharge of roughly 30 spikes/sec

The highest levels routinely presented in the determination of threshold tuning were ap proximately 80–90 dB SPL Early experiments showed that the sensitivity of the ear could change during the course of the experiment if tuning curves were determined repeatedly at higher levels To further minimize the acute sound exposures the characteristic frequency (CF) was estimated audiovisually and the automated tuning curve procedure was initi ated at a frequency just above CF For some units no response could be detected by audio visual criteria for any frequency even at the highest levels Such determinations were double checked in the early experiments with the automated threshold seeking process Since the audiovisual and computerized proce ... always agreed the audiovisual criteria ... were deemed sufficient in later experi to define nonresponsive units

Throughout the experiments the cat s rec tal temperature was maintained near 37 5°C (±1°) In addition the Visual Detection Level (VDL) for the gross neural response to clicks (as recorded from a wire near the round win dow) was monitored Increases in the VDL (i e increases in the level required to elicit a just noticeable response) during the course of an experiment often indicated acute de terioration of the inner ear Data included in this study are from animals in which the VDL at the end of the experiment was within 6 dB of the initial VDL

The gross responses to clicks were also use ful in making a quick appraisal of the normality of the ear before single unit recordings began The difference between the VDL recorded in a noise exposed ear and the normal VDL provided a rough estimate of the degree of single unit pathophysiology It was often pos sible to predict the general pattern of single unit pathophysiology by examining the gross response waveform as a function of click level and polarity The gross response data will not be systematically reviewed here

In each experiment the aim was to obtain a representative sample of units across char acteristic frequencies In most experiments an effort was made to obtain a representative sample of spontaneous discharge rates (SRs) as well This meant that many of the units en countered late in an experiment were pur posely bypassed if data from several units of similar SR and CF had already been obtained Since there were relatively few units with very low SRs (less than 1 spike/sec) such units were typically not skipped and therefore most distributions of spontaneous rates are biased in favor of low rate units In certain exper iments it was important to obtain a nonselec tive sample of SRs in order to determine the nature of the rate distribution To obtain this relatively unbiased sample an effort was made to obtain the SR as well as the tuning curve from every unit encountered in each microelectrode penetration through the nerve regardless of the accumulated sample of units A representative CF sample was obtained by systematically varying the region of the nerve bundle through which the penetrations were made

III HISTOLOGICAL PREPARATION AND EVALUATION OF THE TEMPORAL BONE

All the noise exposed and control ears were processed for light microscopy via serial sec tioning of celloidin embedded specimens The ears of the four chamber raised animals were embedded in epon and examined in the light microscope as a surface preparation (Bohne 1972 Spoendlin & Brun 1974) The celloidin sectioning technique was used for the majority of the ears because it allows an evaluation of all structures of the inner middle and external

ears Although the surface preparation technique is a convenient way to assess the sensory cell populations, it is not as well suited for evaluation of structures such as ganglion cells, stria vascularis, spiral ligament, limbus, Reissner's membrane, etc Furthermore, the *entire* sensory cell population *can* be evaluated in serial celloidin sections

For every temporal bone processed using the celloidin technique, the animal was sacrificed by intravascular perfusion of warm saline followed immediately by Heidenhain-Susa fixative Throughout the embedding procedure, the right and left inner ears were processed together, thus providing a measure of histological control in the side not directly exposed to the sound source After perfusion, the tissue was immersed in fixative for 48 hours at roughly 3°C After fixation, the tissue was decalcified, dehydrated, embedded in celloidin and cut in the horizontal plane into sections 20 μm in thickness A more complete description of the tissue preparation has been published elsewhere (Schuknecht, 1974) Every fifth section was routinely stained with hematoxylin and eosin and mounted for histological evaluation When examination of every fifth section failed to reveal significant hair cell loss, a second set of slides (interleaving with the first set) was stained, mounted and evaluated In cases where the loss of hair cells was very punctate, every section in the vicinity of the lesion was processed and examined

The cochleas were reconstructed as described by Guild (1921) and Schuknecht (1953) Plots of sensory cell loss (cytocochleograms) were generated by determining the percentage of cells remaining in each row of hair cells in a given cross section through the cochlear duct and then averaging these percentages for all sections within each 1% increment of cochlear length Each section was evaluated with a 100× oil immersion objective The number of missing hair cells in each row was ascertained by focusing through the entire 20 μm thickness of the section With practice, such an evaluation could be made for any section, radial or tangential In many cases, the evaluations were done by two observers The results from two trained observers were never significantly different

When every fifth section is evaluated, the condition of the cochlea can be sampled at approximately 140 positions along its length (When a second set is evaluated, the number of locations is doubled) But, due to the spiral shape of the cochlea, the sampling density is somewhat lower in the regions where the cochlea is cut tangentially

The ears prepared for examination via surface preparation were perfused with glutaraldehyde and paraformaldehyde, postfixed in osmium tetroxide, dehydrated, and embedded in epon The entire organ of Corti was then dissected out in roughly 30 pieces The epon in each piece was filed down close enough to the surface of the organ of Corti to allow examination of the hair cells with an oil immersion, 100× objective

In a number of ears, an attempt was made to evaluate the condition of the sensory cells which remained in the cochlea In each cross section through the cochlear duct, the condition of the hair cell outline and cytoplasm was rated as described in Fig 28, and the stereocilia were rated as normal, clumped, or missing A separate rating on each criterion was determined for each of the four rows of sensory cells Included in the anlaysis were 14 ears, 7 normal and 7 abnormal Since it is very difficult to maintain precisely the same criteria from ear to ear when evaluating structures which may be varying in several dimensions, it was important that this analysis of condition be performed with the evaluator "blind" to the physiological results from the ear being evaluated To this end, one person took all the slides from these 14 ears, obscured all marks which would identify the cat, shuffled the slides, and then numbered them consecutively A second person made the evaluations without knowledge of the cat numbers, which were not revealed until the evaluation was completed

Results

I ELECTROPHYSIOLOGY

Two important measures of auditory nerve fiber activity are the tuning curve and the spontaneous discharge rate The tuning curve provides a measure of the sensitivity of single units as a function of frequency Furthermore, the tip of the tuning curve defines a CF which can be used as a physiological measure of fiber location along the cochlear partition The SR is important in that it is strongly related to unit threshold in normal animals and is relatively independent of CF (Kiang et al , 1965, Liberman, 1978)

In order to describe noise induced alterations in these two characteristics, one must first define what is to be used as a baseline for comparison A distinction is drawn between "control" animals and "normal" animals The "control" group consists of animals chosen from those routinely available from local animal suppliers, selected only for clean external ears and generally healthy appearance In most cases, nothing is known about the previous history of noise exposure for these animals Since we define as "normal" those characteristics which are *common* among the control animals, a "control' animal can, in some cases, be "abnormal' It must also be noted that what is 'normal' under this definition may well include *pathological* conditions that are very common Some evaluation of the extent to which such "normal pathology" occurs can be seen when "normal" data are compared with data from young animals born and raised in a low noise environment (Liberman, 1978) Thus, depending on the issue under consideration, the data from the "exposed' ears (i e , those *experimentally* exposed to high level noise) will be compared with either control data, normal data, or the data from chamber raised animals

A. Threshold tuning curves

1 *Properties of tuning curves in control animals*

The tuning curve of an auditory nerve fiber is a plot of its threshold to tonal stimuli as a function of frequency The frequency to which a fiber is maximally sensitive is called the characteristic frequency or CF The shape of the tuning curve in normal animals varies with CF (Fig 1) The tuning curve of a high CF unit has a sharply tuned, sensitive region, the "tip", and a broadly tuned, insensitive region, the "tail', that extends to frequencies well below the CF For a low CF fiber, a tip and a tail are still distinguishable, but the latter can extend to frequencies both above and below the CF For units with CFs near 1 5 kHz, the tip and tail portions are difficult to distinguish by inspection of the tuning curve alone (Kiang, Liberman & Baer, 1977)

As shown in Fig 2, the sensitivity of auditory-nerve fibers in a given animal varies with CF and SR (Kiang et al , 1965) The most sensitive units at each CF are those with high SRs (rates greater than 18 spikes/sec) Within any CF region, all the high SR units in each animal have similar thresholds All the medi

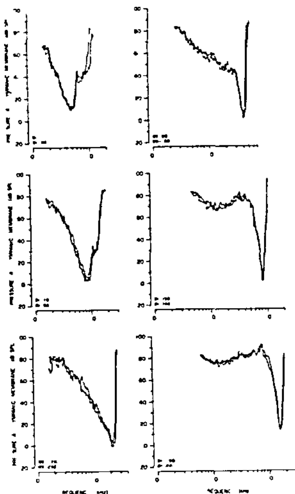


Fig 1 Shapes of tuning curves in six d fferent CF regions These data are selected from two control an mals Each panel contains the tun ng curves of two different units with similar CF from the same animal For these and all subsequent tuning curves the sound pressure level (SPL) is measured at the tympanic membrane (TM) and is ex pressed in decibels (dB) re 0 0002 dynes/cm² The num bers in the corner of each panel identify the cat and unit number for each of the curves S nce units are num bered consecutively in the course of an experiment the numerical separation between two units gives some idea of the elapsed time between the two recordings (approximately 12 hours per 100 units) The fact that the two curves in the bottom right panel were obtained more than 16 hours apart gives some indication of the stability of the aud tory nerve preparat on

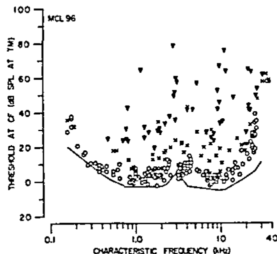


Fig 2 Distribution of thresholds at CF from an animal wh ch was raised from birth in a soundproofed chamber Each data point represents the threshold at CF of a dif ferent unit data from all units obtained in this cat are [illegible]

SR The solid curve superimposed on the distribution is the best threshold curve (BTC) which represents the lowest CF thresholds seen in a sample of more than 4 500 units from 43 animals includ ng exposed and control (but not chamber raised) animals

um SR units are roughly 10 dB less sensitive Low SR units can be as much as 70 dB less sensitive than high SR units of similar CF (Liberman 1978) Among the units in any of the three SR groups, the sensitivity (measured as the SPL at the tympanic membrane) varies with CF Sensitivity is typically maximal for CFs between 1 and 10 kHz and falls off for units with lower and higher CFs There is often a local rise in the threshold distribution of units with CFs between 2 and 4 kHz

The data in Fig 2 are from one of the animals born and raised in a soundproofed chamber The minimum thresholds in this ear were significantly lower than the average thresholds for control animals In fact, the minimum thresholds in most CF regions were near or below the ' best threshold curve ' (BTC) for control animals (solid line) The means and standard deviations of the control thresholds are shown in Fig 3 The deviation of the mean control thresholds from the BTC (and thus from the thresholds seen in chamber raised animals) suggests that most of the con trol animals had some long term damage to the peripheral auditory system by the time the

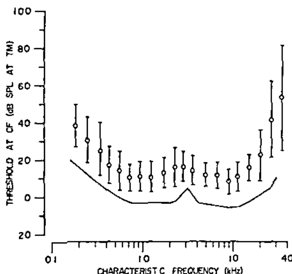


Fig 3 Distribution of CF thresholds for high SR units from control animals The unit sample includes all the high SR units (1848) from 34 control animals The CF continuum has been divided into 20 logarithmically spaced bins Each open circle represents the mean threshold for all the units in the sample with CF in that bin The vertical lines above and below each circle indicate plus and minus one standard deviation The solid curve is the best threshold curve described in the caption to Fig 2

single-unit experiment was performed This distribution of means can be taken as the best guess for the pre-exposure thresholds of the high SR units in our noise exposed animals

In normal ears, the auditory-nerve fibers tonotopically arranged within the nerve dle, i e , a given CF region is more strongly represented in some regions of the bundle than in others (Kiang et al , 1965) This normal tonotopy is illustrated in Fig 4 Based on the location of the electrode penetration (schematized in the central panel of Fig 4), the overall progression of CF with increasing electrode depth is predictable This knowledge is useful in obtaining an adequate sample of units over the entire CF range in each ear and in estimating original CF for units whose tuning properties are so abnormal that no CF can be assigned

2 *Noise-induced abnormalities of the tuning curve*

In noise-exposed ears both normal and abnormal tuning curves are found Some of the abnormal tuning curves observed for high-SR units are shown in Fig 5 All of the tuning curves in this array probably come from units that once had CFs between 2 and 4 kHz For comparison, the tuning curve of a normal, 3-kHz unit is shown at the extreme left

Consider first the tuning curves in the top row of the figure Each of these curves shows a higher-than normal threshold for both the tip and tail segments of the tuning curve At the tuning curve tip, this apparent threshold shift is roughly 20, 40, or 60 dB in the first, second and third columns, respectively This type of abnormality, in which both the tip and tail of the curve seem to have elevated thresholds, will be called the "type-V" tuning curve

A very different abnormality is illustrated in the bottom row of the array These curves also show 20, 40, or 60 dB of threshold shift for the tip, but the tails are *more* sensitive than those of control ears This "hypersensitivity" of the tuning-curve tail can be as great as 30 dB in some cases (Kiang, Liberman & Levine, 1976) This type of tuning-curve abnormality, in which the hypersensitive tail is separated from a hyposensitive tip by a notch, will be called the "type-W" tuning curve

In the middle two rows of this array the tuning-curve shapes are intermediate in tail sensitivity between the two extremes just described Arranging the abnormal tuning curves in this array demonstrates that *different degrees of threshold shift on the tuning* curve tail (vertical dimension of the array) can be seen for each level of threshold shift at CF (horizontal dimension of the array)

The conclusions derived from this array of tuning curves depend upon the assumption that all the curves were from units which were initially from roughly the same CF region Several of the curves are so abnormal that there is some question as to how reliably the original CF can be estimated In the case of unit 45-84 (bottom right-hand corner), the frequency to which the unit is maximally sensitive is near 500 Hz However, the 500-Hz point appears to be on the tail of the tuning curve instead of being at a sharply tuned tip

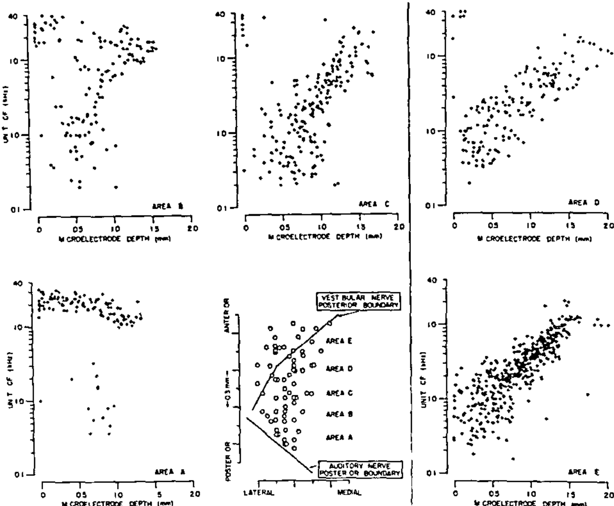


Fig. 4. Tonotopic organization in the auditory nerve. The data in this figure are from 72 microelectrode penetrations through the auditory nerves of 15 different normal animals. The entry point for each of these penetrations as it appeared through the operating microscope during the experiment is marked on a schematic drawing of a left auditory nerve (center panel, bottom row) as seen from a posterior fossa approach. A description of the way in which data from different ears were aligned with respect to each other is given in the Methods. The five panels surrounding the schematic drawing display the CF and relative depth (in the penetration) of all the units encountered in all the microelectrode passes through each of the five areas designated in the central drawing. The relative position of each unit within the pass is determined from the reading on the micrometer used to advance the electrode. These data are qualitatively consistent with the anatomical study of the auditory nerve projections described by Sando (1964).

We have chosen to assign CF not according to the maximum overall sensitivity but according to the maximum sensitivity in the region of sharpest tuning. Thus, CF is found by locating (for both normal and abnormal units) the most sensitive point on the tuning curve in the region where the rate of change of the slope is the greatest.

There are several reasons to believe that this method provides a reasonable estimate of the original CF of an abnormal unit. One line of evidence is derived from traumatized animals in which the majority of tuning curves appear normal. Data from one such animal are shown in Fig. 6. After CFs are assigned and the tuning curves arranged in order of increasing CF, there is a smooth progression from normal units to abnormal units and back

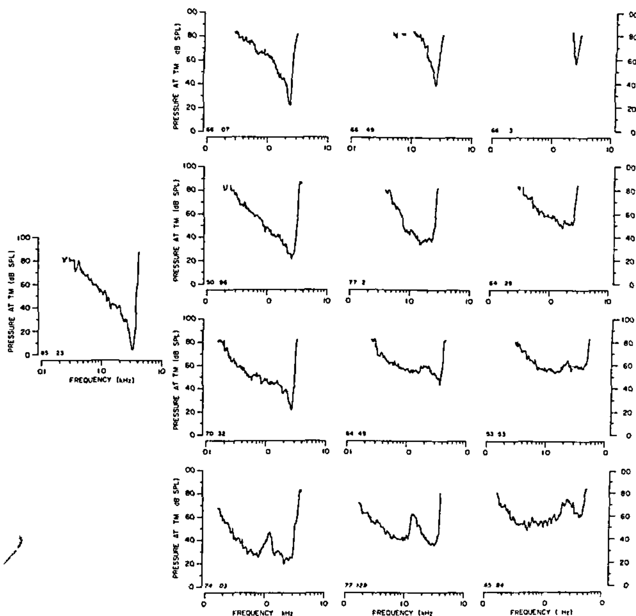


Fig 5 Abnormal tuning curves of 12 high SR units with CFs between 2 2 and 4 0 kHz from nine exposed animals A normal tuning curve from a sensitive high SR unit with CF of 3 2 kHz is shown at the left for comparison The abnormal curves are selected from the total collection of data from high SR units in this CF range They are arranged so that the threshold at CF increases towards the right and the threshold on the tuning-curve tail increases towards the top

to apparently normal units All abnormal curves are restricted to one CF region (dotted lines), and there is no intermingling of normal and abnormal units Another line of evidence is derived from the tonotopic organization of the auditory nerve described above Consider the case of MCL 62 (Fig 7) a traumatized cat showing severe threshold shifts for units in the mid-frequency region When CF is assigned as described above, all the abnormal units fall within the CF region encompassed by the dotted lines Note the orderly pattern of thresholds at CF The progression of unit CF with increasing microelectrode depth for each of two penetrations through the nerve of this traumatized animal is shown Each should be compared with the normal CF progression (shown in Fig 4) for the appropriate region of

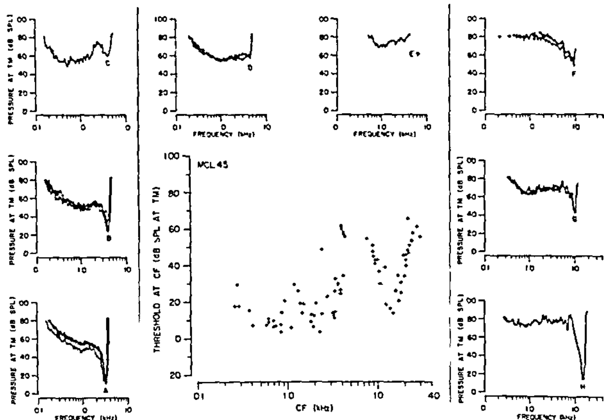


Fig 6 CF threshold plot and selected tuning curves from one acoustically traumatized animal The dotted lines in the threshold plot indicate the CF region within which the thresholds at CF for the high SR units were more than one standard deviation from the mean of the normal data Each of the panels around the threshold plot contains the tuning curve of one or more unit(s) and a letter placed in the frequency region of the CF The tuning curves labeled A through D have CFs on the low frequency side of the abnormal CF region the tuning curves labeled F through H have CFs on the high frequency side The unit whose tuning curve is shown in panel E was one of the two units obtained in this animal which had tuning so broad that no CF was assigned The SRs of all the units for which tuning curves are illustrated were greater than 20 spikes/sec except for the two medium-SR units (7 6 and 4 4 spikes/sec) whose curves are shown in panel D

the nerve In each case, the CFs assigned to the abnormal units appear to be reasonable

Thus far only the tuning curve abnormalities in the mid CF region have been described An array of tuning curve abnormalities analogous to that in Fig 5 can be constructed for high CF units in noise exposed ears (Liberman 1976) The types of tuning curve shapes encountered among the abnormal low CF units are shown in Fig 8 Note the tuning curve shape of the normal unit (pictured on the left) If, for low CF units, the broadly tuned tail is considered to extend normally to frequencies above rather than below the CF (Kiang Liberman & Baer, 1977), the kinds of tuning curve alterations seen for the low CF units are comparable to those seen for the mid and high frequency units The three units whose tuning curves appear in the top row of Fig 8 have a steep high frequency slope with little sign of the tail which should extend to frequencies above CF At the other extreme, in the bottom row, the curves show prominent high frequency tails, separated from the tip region by small clefts

Some units from abnormal animals had tuning curves so broad that no CFs were assigned (Fig 9) Most units with such bowl shaped tuning curves had very high threshold, but some (such as unit 43 88 or 76-124) responded

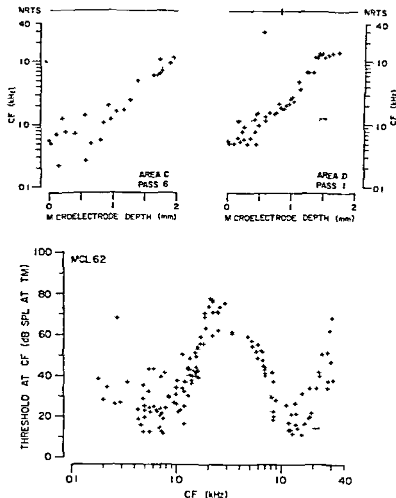


Fig 7 CF vs depth of penetration in two microelectrode passes through the auditory nerve of an acoustically traumatized animal (top panels) The bottom graph shows the thresholds at CF for all the units encountered in this experiment The dotted lines in each of the three panels designate the CF regions where the thresholds at CF for the *high SR units were more than 1 standard* deviation from the mean of the normal data The location in the penetration of any unit with no response to sound is designated by a vertical mark on the horizontal line labeled NRTS (Only one nonresponsive unit was seen in these two passes) The area designated for each pass refers to the areas of the nerve described in Fig 4

to tone bursts at levels as low as 40 dB SPL One way to estimate the original CF of such units might be to assume that the bowl shaped *functions represent the tails of tuning curves* for which the sharp tip has been completely eliminated However, as illustrated in Fig 9, many of these bowl-shaped curves do not match the tails of normal tuning curves from any CF region Presumably, the abnormal curves are hypersensitive

All of the tuning curves shown in Figs 5, 8, and 9 were those of abnormal high-SR units The shape changes seen for the abnormal medium-SR units were quite similar All the abnormalities just described, including tail hypersensitivity can be found among the medium-SR units from noise-exposed ears Furthermore, within each ear, medium SR units typically show the same types of tuning-curve abnormalities as the high-SR units in the same CF regions (Fig 10) Note, however, that in the sound-exposed animals the normally strict *relation between SR and threshold (Liberman,* 1978) is not always maintained

Among the abnormal low-SR units from acoustically traumatized animals, there seems to be less diversity of tuning-curve shapes Type-W tuning curves and tail hypersensitivity were virtually never seen among the low-SR units However, as our sample of abnormal low-SR units was much smaller than that of high- and medium SR units, this observation must be treated with some caution In a few instances, low-SR units have shown a different tuning-curve abnormality than was seen for the high-SR units of similar CF One such case is illustrated in the bottom panel of Fig 10

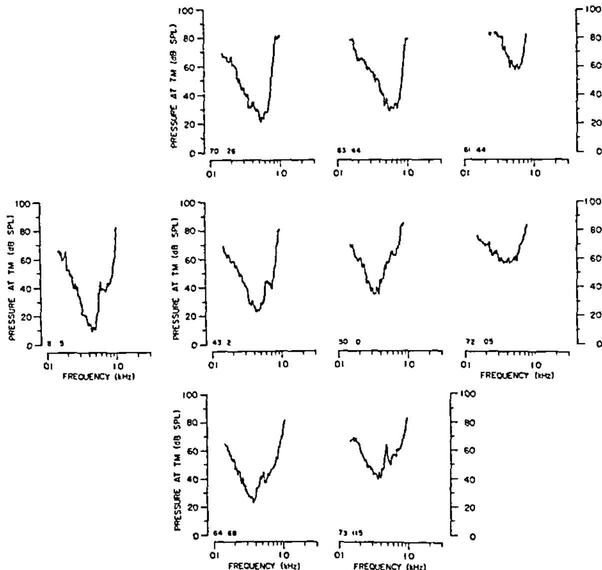


Fig 8 Abnormal tuning curves of eight high SR units with CFs between 0 32 and 0 56 kHz from eight traumatized animals One normal tuning curve from a high SR unit with CF at 0 5 kHz is shown at the left The figure is arranged as was Fig 5

3 *Patterns of tuning curve abnormalities in individual cases*

One way to illustrate the pattern of abnormality in a given ear is to plot the distribution of thresholds at CF for all the units obtained Such CF threshold plots are shown in Figs 11, 12, and 13 for nine different noise exposed animals

In each of the four cases illustrated in Fig 11 threshold abnormalities were seen only in a narrow range of CF As the frequency of the traumatizing stimulus increases, the abnormalities appear to be more restricted in the CF dimension (using a logarithmic frequency scale) In ears exposed to 1 5, 3 0, or 6 0 kHz, the CF regions showing maximal threshold elevation were close to the spectral peak of the traumatizing stimulus (as represented by the arrows) Each of the six cases of restricted abnormalities associated with the noise bands at 1 5, 3 0, or 6 0 kHz showed similarly good agreement between the frequency components

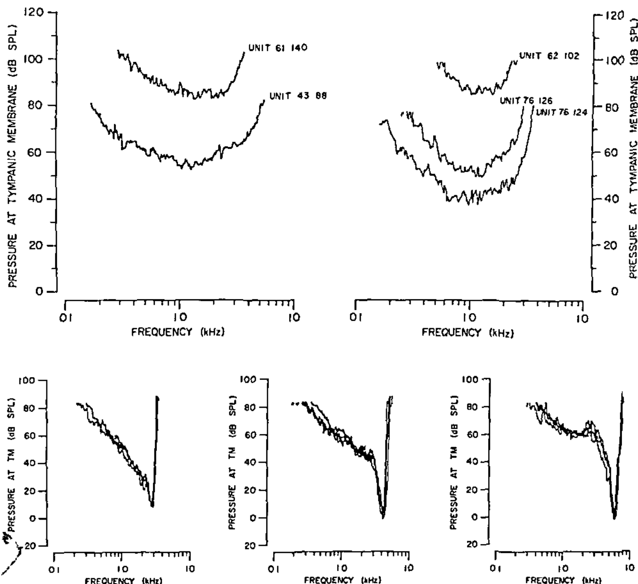


Fig 9 Tuning curves for five units with indeterminate CFs from four traumatized animals (top row) In the bottom three panels the tuning curve for the most broadly tuned unit 43 88 is compared with the tuning curves for high SR units from three different CF regions in the normal ear MCL 85 The SRs of units 61 140 43 88 62 102 76-126 and 76-124 were 38 8 13 9 141 38 9 and 41 4 spikes/sec respectively

of the stimulus and the CF region with maximally elevated thresholds at CF In the ear exposed to the high frequency noise MCL 68, the abnormal CF region was centered at 18 kHz, 1/2 octave higher than the spectral peak of the noise band Since this was the only case of a restricted abnormality at high CF, it is not clear whether this discrepancy is significant It is possible for example that the abnormality in this ear was a preexisting one that was unrelated to the experimentally controlled noise exposure

In some other cases exposed to narrow band stimuli, the pathophysiology was less restricted than that seen in Fig 11 When the threshold shifts were more extensive, the abnormalities always involved the high CF regions to a greater degree than the low Each of the animals whose CF threshold plot is shown in Fig 12 was exposed to a narrow

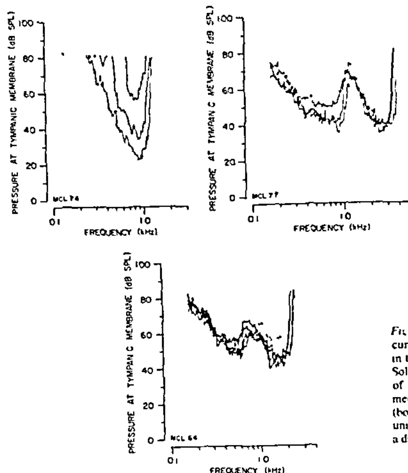


Fig. 10 Comparison of abnormal tuning curve shapes for units with different SRs in the same CF region of the same animal. Solid curves represent the tuning properties of high SR units, dotted curves represent medium SR units, and the dashed curve (bottom panel only) was from a low SR unit. Each of the panels displays data from a different abnormal animal.

band noise centered at 6 kHz. In the case exposed to the highest intensity, MCL 73, no responsive units with CFs greater than about 2 kHz could be found.

When wideband stimuli such as low-pass noise were used, a different pattern of threshold abnormalities was created. In both cases shown in Fig. 13, there were large threshold shifts for the low CF units. No units were found with CFs in a mid-frequency band, and there was relatively less effect on the high CF units. In each case, the envelope of minimum unit thresholds was similar in shape to the spectrum of the trauma stimulus.

In cases for which there is a large gap in the distribution of CF (as in Figs. 12 and 13), there is some question as to whether the gap reflects incomplete sampling of the fibers in the auditory nerve, or whether one can assume that there really were no units to be found in that CF range. Knowledge of the normal tonotopic organization of the nerve helps in searching specifically in those regions of the nerve where the "missing" units should be found. Data from several microelectrode penetrations through the nerve of one severely traumatized animal are shown in Fig. 14. Comparison of the data from these three passes with the pooled normal data in Fig. 4 suggests that most of these units without response to sound must once have had CFs in the high and medium frequency range. Thus, the failure to find units with these CFs in this particular animal is probably not due to incomplete sampling.

Thus far, only the patterns of threshold shift at CF have been described. The patterns of threshold abnormalities on the tuning curve tails are also of interest. Some of the important

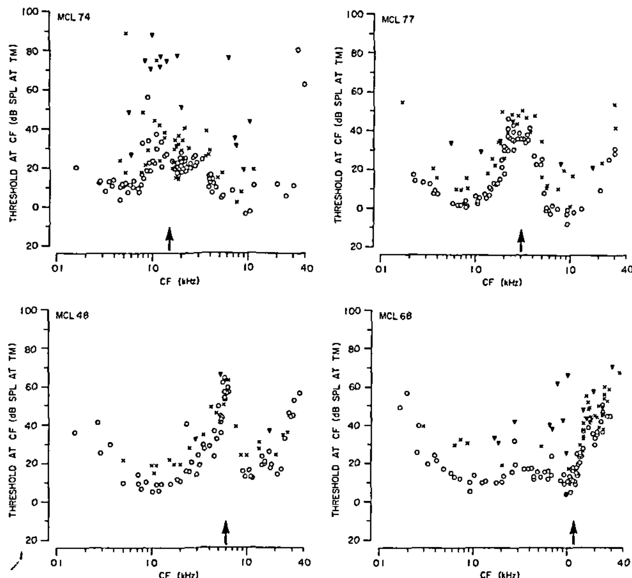


Fig 11 CF threshold plots for four animals exposed to narrow band noise The arrows are positioned at the center frequency of the noise exposure for each animal In MCL 74 electric shocks were used while advancing the m croelectrode in search of single units If an acoustic search stimulus had been used as in the other three cases the cluster of units with very high thresholds and very low SRs m ght not have been found

features of those patterns are illustrated in Fig 15 The six panels trace the changes in tuning curve shape (seen in the data from one ear) throughout the CF region where CF thresholds appeared to be significantly ele vated At the CF region near 0 6 kHz (panel A), the tip thresholds are similar to those from the chamber raised animal but the high fre quency tails of the curves appear hyposen sitive As the tip thresholds are elevated (panel B), the shift in the tuning curves seems nearly symmetrical, but there is a hint of the tail hy persensitivity which is more apparent for units with a somewhat higher CF (panel C) At roughly the middle of the CF range of abnor mal units (panel D) the tuning curves are type W with similar thresholds on the tip and tail of the tuning curves The most striking tail hypersensitivity in this ear was seen among the units on the high frequency border of the abnormal region (panel E) Note that the tip thresholds here are only slightly higher than

those seen in the chamber-raised animal while the tail thresholds are some 25 dB lower At a slightly higher CF (panel F), the tuning curves are virtually identical to those from the chamber-raised animal

Not every abnormal case showed the same progression of tuning curve shapes Both hypersensitivity and hyposensitivity of the tuning-curve tail were not always present However, when tail hyposensitivity was present, it was always found in units on the low frequency side of the abnormal region When tail hypersensitivity was present, it was always most striking on the high frequency side Almost every abnormal case showed at least one CF region where the tails of the tuning curves were significantly more sensitive than those of units with similar CF in normal animals Across all abnormal cases, tail hypersensitivity could be seen in virtually any CF region It is interesting to note that the tails of units from "normal" cases were hypersensitive relative to those seen in chamber raised animals (Liberman, 1978)

B. Spontaneous activity

1 *Time pattern and rates of spontaneous discharge in normal animals*

The important features of spontaneous activity in normal animals have been described in previous publications (Kiang et al, 1965, Walsh et al, 1972, Liberman, 1978), so only a brief summary will be presented here Virtually every fiber contacted in the auditory nerve will discharge spontaneously in the absence of controlled acoustic stimulation Histograms of the time intervals between adjacent pairs of spontaneous spikes show a mode of less than 10 msec and a roughly exponential decay from the mode The SR in different fibers ranges from near 0 to over 100 spikes/sec The distribution of rates is fundamentally bimodal, with one cluster of units having rates between 40 and 100 spikes/sec and a second having rates less than 10 spikes/sec (Fig 16) The rate

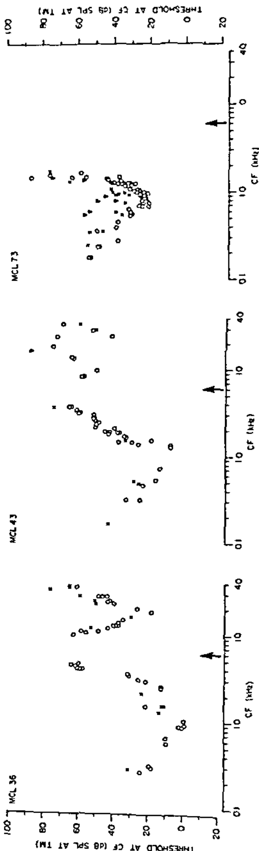


Fig 12 CF threshold plots for three animals exposed to narrow band noise centered at 6 kHz The bandwidth and intensity of the traumatic stimulus were different in the three cases (Table I) The paucity of low SR units in MCL 36 and MCL 43 is due to the deliberate selection of units during the experiment

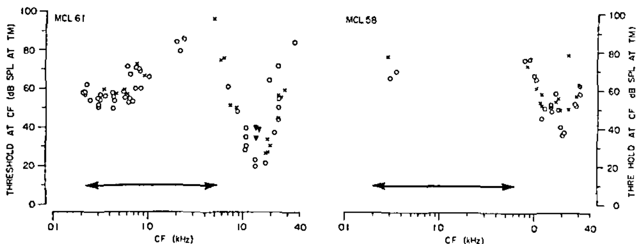


Fig 13 CF threshold plots for two animals exposed to low pass noise The arrows indicate the bandwidth of the traumatizing stimulus 20 dB down from the spectral peak at 2 kHz The paucity of low SR units in these animals was *not* due to any conscious selection of units

NRTS
CF (kHz)
AREA A PASS 4
AREA C PASS 2
AREA E PASS 5
MICROELECTRODE DEPTH (mm)

MCL 73
THRESHOLD AT CF (dB SPL AT TM)
CF (kHz)

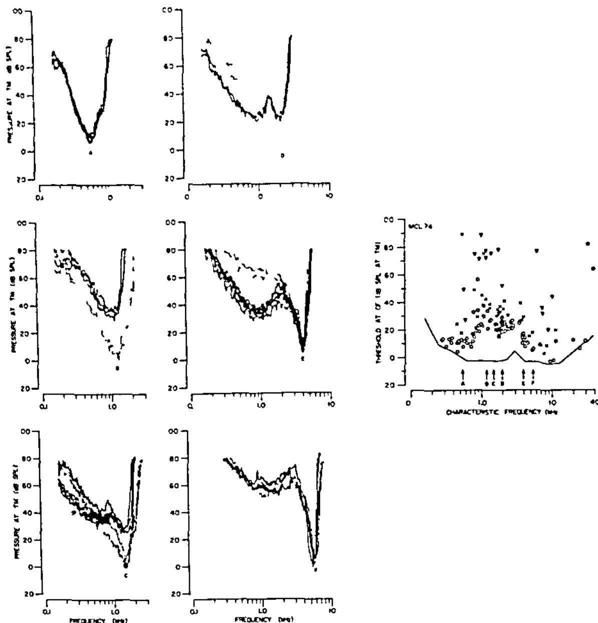


Fig 15 Abnormalities of the tuning-curve tail in several CF regions from one traumatized animal Each of the six panels at the left displays the tuning curves (solid lines) of several high SR units from a different CF region of MCL 74 The stippling in each panel represents the tuning-curve data from high SR units in one of the chamber raised animals The letter in each panel serves to identify the CF region with respect to the distribution of CF thresholds for this ear shown at the extreme right

Fig 14 CF threshold plot and CF progression for three passes through the auditory nerve from one acoustically traumatized animal The conventions for data display are as described for Fig 7 except that the nonresponsive units are represented differently the shorter vertical lines (through the horizontal line labeled NRTS) designate those nonresponsive units which showed no spontaneous discharge in a 30-second time sample

distribution is similar throughout the entire CF range in those chamber raised animals with exceptionally low single-unit thresholds (Liberman, 1978) In normal control animals, however, the distribution often appears compressed for the CF region above 10 kHz, very few high CF units show rates above 40 spikes/

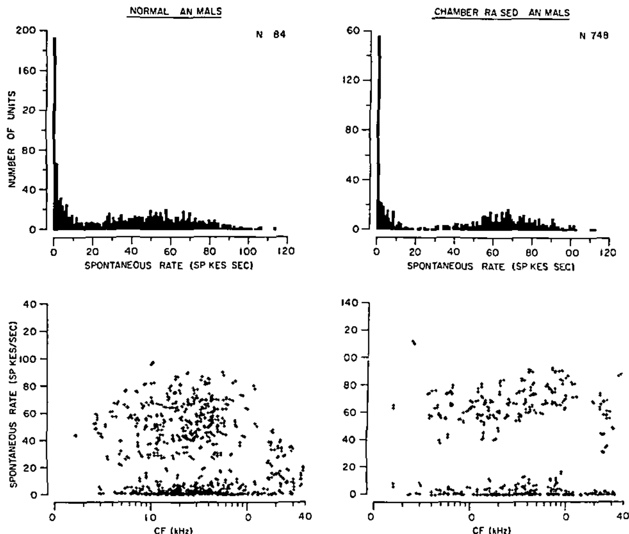


Fig 16 D stribution of spontaneous d scharge rates for ormal and chamber raised an mals Included on the left side are all the data from 11 normal control an mals on the right side are the data from 3 chamber ra sed an mals *n* refers to the number of units in the sample The b n width for the h stograms is 1 spike/sec

sec and an inordinately high proportion of units shows rates between 10 and 40 spikes/sec (Fig 16) This compression of the rate distribution at high CF may reflect a patho logical condition in the normal animals

2 *Noise induced abnormalities of spontaneous activity*

The data from noise exposed ears suggest that acoustic trauma can alter the time pattern of spontaneous discharge In Fig 17 the interval histograms for three abnormal high SR units can be compared to that for one normal unit of similar SR These data are meant to illus trate the general rule that in a noise exposed ear most of the units which still respond to sound retain a normal distribution of spike intervals for spontaneous activity Only among units with severe threshold shift is one likely to find abnormal interval histograms of spontaneous discharge For unit 72 33 (right panel Fig 17) the threshold shift was prob- ably on the order of 70 to 80 dB the interval histogram shows a higher peak at the low in tervals and the decay from the mode is faster than normal This type of abnormality in the discharge pattern is clearly audible in the spike train which tends to sound bursty

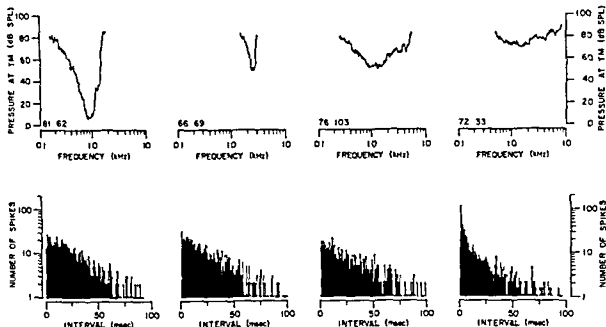


Fig 17 Interval histograms of spontaneous activity and tuning curves from one normal (left) and three abnormal high SR units Each interval histogram displays the number of occurrences (on a logarithmic vertical scale) of specified time intervals between adjacent spikes (horizontal axis) The samples of spontaneous activity were 30 seconds long Bin width on each histogram is 500 microseconds The average rates of spontaneous discharge for these four units were from left to right 41 spikes/sec 40 spikes/sec, 32 spikes/sec, and 37 spikes/sec

Abnormal time patterns of spontaneous discharge are much more common among units that have lost their response to sound (Fig 18) Although some nonresponsive units show a normal time-pattern of discharge (see top row), the tendency in severely abnormal units is for the spontaneous discharge to occur in bursts separated by long periods of silence In the case illustrated in the bottom row, the discharge pattern consisted of short-interval, doublet or triplet spikes We recorded from this particular unit for over six minutes, and the bursty discharge was maintained throughout this time period Most of the nonresponsive units in traumatized animals have bursty patterns of spontaneous discharge or no spontaneous activity at all

If acoustic trauma can alter the time pattern of spontaneous discharge, can it also alter the average rate? Since in data from exposed ears, the SRs are generally within the range seen in normal cases, the answer to this question can only be found by considering the distribution of SRs in large samples of units The distribution of rates for all units obtained in noise-exposed ears is shown in Fig 19 The data from these cats have been divided into three groups In the bottom row, the distribution of SRs is shown for those units which gave no response to sound In the top row, data are included only from CF regions in which the high SR units were all within 20 dB of the BTC. The middle row contains data from units in CF regions for which minimum thresholds were more than 20 dB from the BTC

The SR distribution for the low threshold CF regions appears to be bimodal at all CF regions In this respect, the distribution is similar to that of the chamber raised animals, suggesting that if the acoustic trauma has not significantly raised single unit thresholds, the SRs of those units have not been altered

The distribution of SRs for the CF regions in which the units appeared to show threshold elevation is significantly different There is an

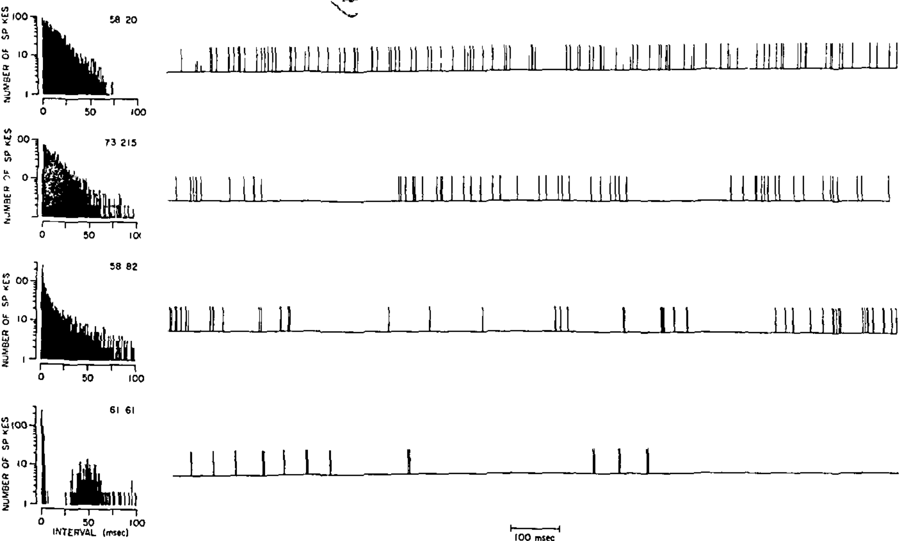


Fig 18 Interval histograms and sample spike trains of spontaneous activity for four nonresponsive units The interval histograms (bin width 500 microseconds) are based on 60 second samples of spontaneous discharge The spike train associated with each histogram displays the time pattern of spontaneous spikes (each spike represented by a vertical deflection) occurring in a 1 5 second sample of the spontaneous run Some of the thicker deflections in the bottom rows represent multiple discharges which are not resolvable on this time scale

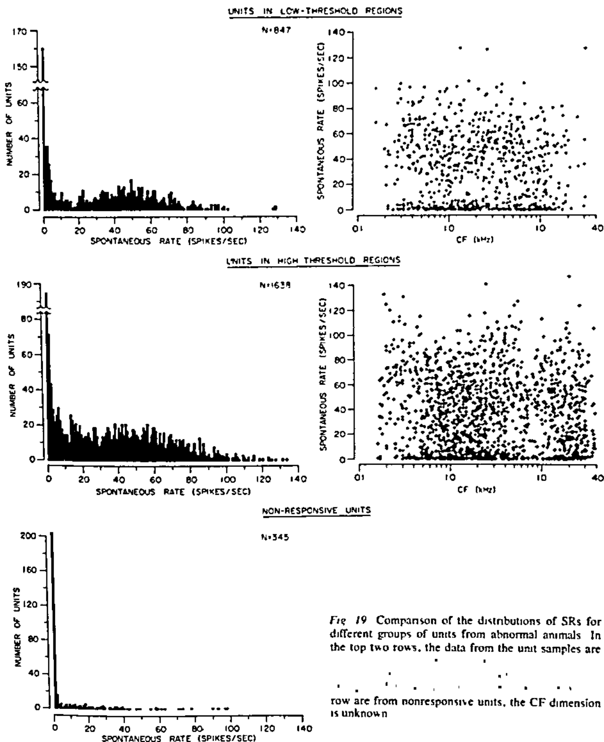


Fig. 19. Comparison of the distributions of SRs for different groups of units from abnormal animals. In the top two rows, the data from the unit samples are

row are from nonresponsive units, the CF dimension is unknown.

increase in the proportion of units with SRs between 10 and 30 spikes/sec. From the scatter plot at the right, it can be seen that this range of SRs was especially common in two CF bands. For the CF region above 10 kHz, it is "normal" to see a high percentage of units with SRs between 10 and 30 spikes/sec (Liberman, 1978). Although this may reflect cochlear

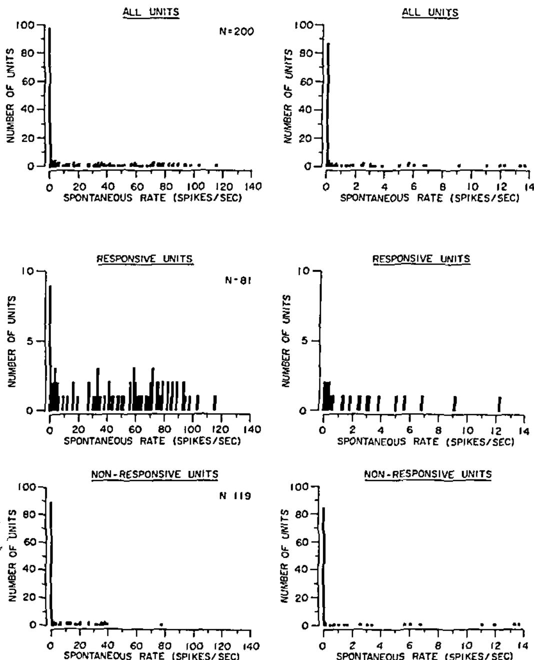
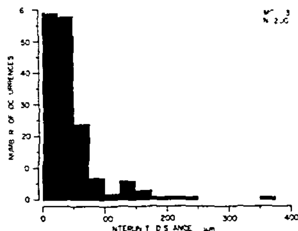


Fig 20 Sample of SRs from one abnormal ear with a large gap in the distribution of CF (MCL 73) The three histograms on the left display the data with a bin width of 1 spike/sec, those on the right show the distributions for the low rate units with a bin width of 0 1 spike/sec The top histogram in each column displays data from all the units obtained from this animal The middle histograms show the rate distribution only for those units which were responsive to sound The bottom histograms contain only the rate data from nonresponsive units In the auditory nerve experiment on this animal, electric shocks were used as the search stimuli while advancing the microelectrode

pathology, this pathology could well have been present in the animals before the noise exposure and thus cannot be said to be noise-induced. It is unusual, however, to see such a high proportion of units in the mid-CF region with rates between 10 and 30 spikes/sec These data suggest that acoustic trauma can alter the distribution of SRs, at least in some CF regions

The same conclusion is suggested by the



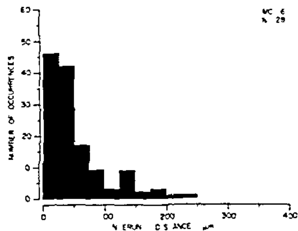


Fig. 21 Density of single units in the auditory nerve from a normal and an abnormal ear. The abnormal animal (left panel) is the same case illustrated in Figs. 14 and 20. Each histogram displays, with a bin width of 25 microns, the distribution of interunit distances seen across all the microelectrode penetrations in a given case. The interunit distances are based on readings of the micrometer used to advance the electrode.

data in the bottom row of Fig. 19. Among the nonresponsive units there is an inordinately high proportion of low rate units and very few units with rates above 40 spikes/sec. Evidence has already been presented that in a noise exposed ear groups of units which have lost their response to sound probably once comprised well defined CF regions (Fig. 14). Since no normal CF region has a distribution of SRs like that for the nonresponsive units, the data suggest that acoustic trauma alters the SR distribution from severely damaged regions of the cochlea.

This noise induced alteration of the rate distribution may be interpreted in one of two ways: either (1) the trauma has altered the SRs of many auditory nerve fibers, or (2) the trauma has resulted in selective disappearance of the units within certain ranges of SR. Data on the density of units within the nerve bundle of abnormal ears suggest that the second explanation cannot be solely responsible for the observed alteration in the rate distribution. Consider the data in Figs. 20 and 21. In an unbiased sample from one abnormal ear with a large CF gap, 43% of the units encountered had SRs below 0.1 spikes/sec (Fig. 20) in comparison to a normal value of roughly 7%. The histograms in the bottom two rows of the figure illustrate that this overabundance of low rate units was due almost entirely to the sample of nonresponsive units. If this sixfold increase in the proportion of very low SR units was due to selective elimination of the units with higher rates, the distribution of interunit distances must surely be affected. One would expect to see an appreciable shift in the distribution toward larger interunit distances. As can be seen in Fig. 21, the unit density was virtually identical to that in a normal ear. Taken together, these observations suggest that when the damage to a region of the cochlea is so severe that the majority of the fibers innervating that region no longer responds to sound, the spontaneous activity of most of these fibers is significantly depressed.

C. Correlating abnormalities of tuning curve and SR

In the previous section it was noted that a noise induced increase in threshold at CF was in some ways correlated with alterations in the SR distribution (Fig. 19). In this section

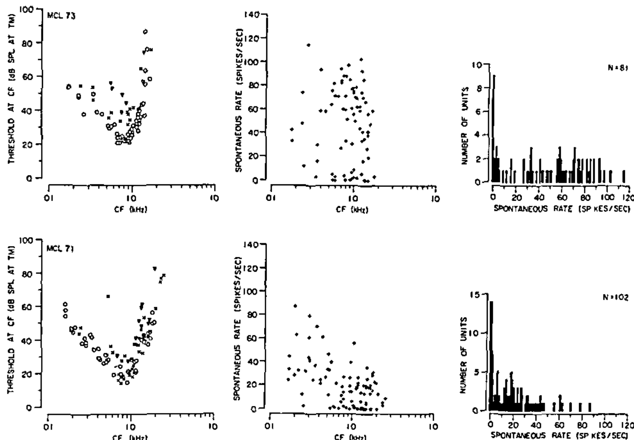


Fig 22 CF threshold plots SR plots and distributions of SRs for each of two traumatized animals The bin width for the histograms is 1 spike/sec In both ears units were sampled in a nonselective way

the nature of these correlations will be examined more closely Data on threshold and SR are compared in Fig 22 for two animals ex posed to similar traumatic stimuli Note that although the plots of threshold at CF are similar in the two animals, the distributions of SRs are significantly different The rate dis tribution in MCL 73 is within normal limits, the distribution for MCL 71 shows a decrease in the proportion of high-SR units and a large increase in the proportion of units with SR between 10 and 40 spikes/sec The plot of SR vs CF reveals that the compression of the rate distribution is most significant for units with CF in and around the region where the thresholds are most abnormal

Another difference between these two animals is seen in the relationship between SR and threshold at CF In MCL 73 this relationship is similar to that in normal animals At any CF region below 1 5 kHz, the high-SR units are the most sensitive, the low-SR are the least sensitive, and the medium SR units have intermediate thresholds This relationship clearly breaks down in MCL 71 Around the 1-kHz region, for example, the high- and medium SR units have similar threshold at CF It was generally true that the relationship between SR and threshold at CF was closer to normal in CF regions where the distribution of SR was closer to normal

In individual cases, the nature of the alterations in the SR distribution was correlated with the nature of the abnormalities in tuning-curve shape If, at a particular CF region in a given ear, there was a significant shift in the character of the SR distribution, there were usually changes in tuning-curve shapes as well An example of this correlation is shown in Fig 23 In this case, there was a significant

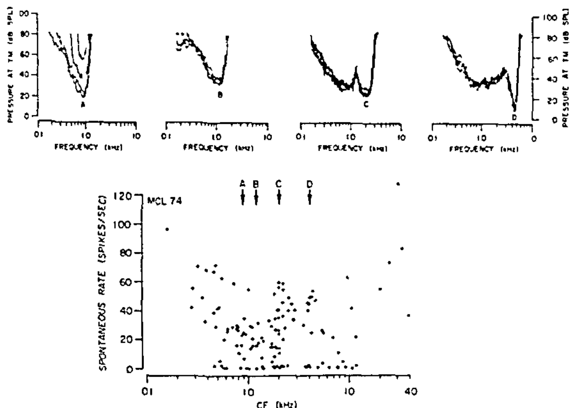


Fig 23 Distribution of SR vs CF in one abnormal animal (MCL 74) shown with selected tuning curves The SR plot contains data from all the units obtained in this animal Each of the panels above it contains the tuning curves of three or more high SR units with similar CF Each of the four arrows on the SR plot denotes the CF region for the tuning curves in the panel with the same letter

compression of the SR distribution in the CF region from 0 8 to 2 0 kHz As shown in panels A and B, the tuning curves in this region were predominantly type V For CF above 2 kHz, the mean SR increased, the percentage of units with SR between 10 and 20 spikes/sec decreased, and type W tuning curves were found (panel C)

In general, the compression of the SR distribution was associated with the type-V abnormality This correlation was evident in the data from Fig 23 A second example, from a different abnormal ear, is shown in Fig 24 In this case, it was the CF region between 2 and 3 5 kHz which showed a compression of SRs out of 35 units in this region, the maximum SR was 50 spikes/sec, and 75% of the units had SRs between 10 and 40 spikes/sec, compared to a value of 6% in the data from chamber raised animals The tuning curves associated with this region were exclusively type V, several of these curves are shown in panel B

Although the compression of the SR distribution was more common, there was evidence in at least two animals that the mean SR could be increased as a result of acoustic trauma Data from one such ear are shown in Fig 25 The distribution of SRs in MCL 76 for units with CF greater than 1 5 kHz seemed shifted to high rates The mean rate was close to 80 spikes/sec More than 25% of the units had SRs over 90 spikes/sec (as compared to the 4% seen in the data from chamber raised animals), and almost no units had rates less than 20 spikes/sec For CF above 1 5 kHz, the abnormal tuning curves were, almost exclusively, type W Some of these type-W curves are illustrated in panels C and D Note that for CF below 1 5 kHz the SR distribution

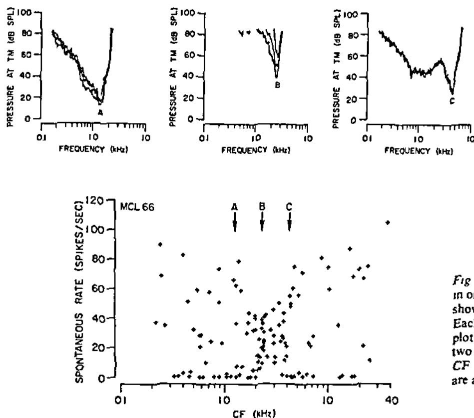
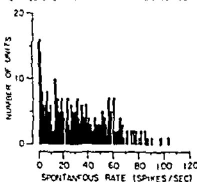


Fig 24 Distribution of SR vs CF in one abnormal animal (MCL 66) shown with selected tuning curves Each of the panels above the SR plot contains the tuning curves of two or more high SR units of similar CF *All other display conventions* are as described for Fig 23

Fig 25 Graph of SR vs *CF* and selected tuning curves from one abnormal animal Each panel above the SR plot contains the tuning curves of three or more high SR units from this animal All display conventions are as described for Fig 23



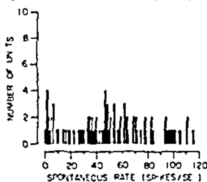


Fig 26 Histograms of SR for CF regions with type V or type W tuning curves The data are pooled from all abnormal ears All units from any CF region in which the high SR units had type V or type W tuning curves are included in the left or right histograms respectively The bin width for each histogram is 1 spike/sec

was somewhat compressed, and the tuning curves were type V

If data are pooled from all traumatized ears, distributions of SRs can be constructed for all CF regions having exclusively type-W or type-V tuning curves (Fig 26) Both distributions are abnormal, but in different ways That for the type W regions shows too few units with rates less than 1 spike/sec The rate distribution for the type-V regions has lost the bimodal appearance and shows an abnormally high proportion of units with rates between 10 and 40 spikes/sec

II HISTOPATHOLOGY

The histological analysis included structures of the external, middle and inner ears Clear signs of histopathology were seen only in the cochlea and auditory nerve No histopathological signs were observed in the tympanic membrane or the ossicular chain

A. Cochlear pathology

Within the cochlea, the histopathological signs were often restricted to the structures of the organ of Corti The sensory cells, especially the outer hair cells (OHCs), appeared to be especially vulnerable Often, there was scattered or even complete loss of OHCs in regions where the inner hair cells (IHCs) were apparently intact (Fig 27, panel B) It was rare to find IHC loss without concomitant loss of OHCs The three rows of OHCs often showed similar patterns of degeneration When differences existed, the first row of OHCs typically showed the most extensive damage

The hair cells that remained in noise-exposed cochleas showed considerable variation in the regularity of outline and/or darkness of staining Fig 28 shows cross sections through several OHCs from two different ears Some of the cells have round outlines with cytoplasm that stains only faintly (see cells labeled "1"), others have irregular cell outlines ("2"), and still others have a darkly stained cytoplasm and a shrunken appearance ("3") Those cells marked "4" show severe pyknosis and crenation Shrunken OHCs were less common in normal ears than in the abnormal ears In making this observation, account was taken of the fact that similar pyknosis and crenation are seen in *all* the sensory cells when the ear is poorly fixed Poor fixation is usually due to inadequate perfusion, which can be readily ascertained because the vessels of the cochlea are filled with blood cells

Some of the IHCs in abnormal ears showed abnormalities of the stereocilia The abnormal IHC shown in Fig 29 (right panel) was from a case sacrificed approximately one year after

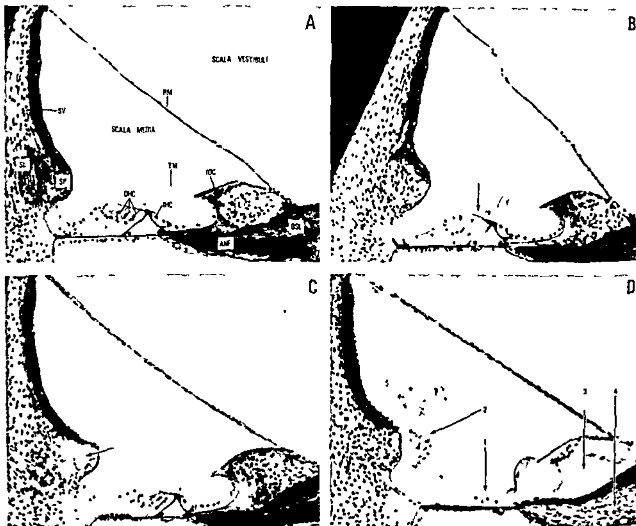


Fig 27 Photomicrographs of cross sections through the cochlear duct in four different ears *Panel A* A cross section from a control ear at a cochlear location roughly 55% of the distance from the base The structures identified by the abbreviations are ANF, auditory nerve fibers, IDC, interdental cells, IHC, inner hair cell, L, limbus, OHC, outer hair cells, OSL, osseous spiral lamina, RM, Reissner's membrane, SL, spiral ligament, SP, spiral prominence, SV, stria vascularis, TM, tectorial membrane *Panel B* Cross section from a noise exposed ear (MCL 76) at a cochlear location 57% of the distance from the base The arrow points toward the location where the OHCs should have been *Panel C* Section through a noise-exposed ear (MCL 27) at a cochlear location 38% of the distance from the base The arrow indicates the region of acellularity in the spiral prominence *Panel D* Section through a noise exposed ear (MCL 71) at a cochlear location 38% of the distance from the base This ear had the longest survival time of any in the study The numbered arrows point toward the following abnormalities 1) organ of Corti replaced by undifferentiated cell mass, 2) cellular debris remaining in scala media almost a year after exposure, 3) acellularity of the central zone of the limbus, 4) degeneration of peripheral axons of auditory nerve fibers The details of noise exposures and survival times for the three abnormal ears can be found in Table I

exposure to high-level noise A complete picture of the abnormalities cannot be displayed in the single focal plane of the photograph As the focus is moved, the normal IHC on the left showed an uninterrupted progression of many fine stereocilia oriented at right angles to the cuticular plate In the example pictured on the right of Fig 29, there appeared to be only a single large stereocilium on each IHC There are several reasons to believe that this phenomenon is the same as that described as "clumped stereocilia" in the electron-microscopic studies of cochlear pathology (Lindemann & Bredberg, 1972; Ades et al , 1974,

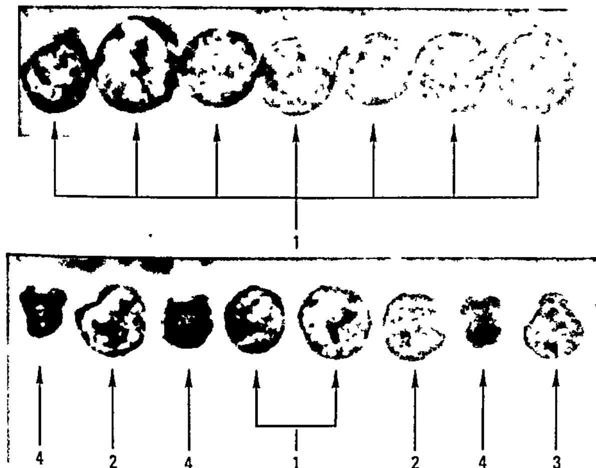


Fig 28 Photomicrograph of cross sections through the cell bodies of OHCs illustrating the criteria by which the condition of sensory cells was rated The number associated (via arrow) with each cell is the rating which was assigned to it in the quasi blind analysis See text for further explanation

Spoendlin, 1976) First, the phenomenon was observed mainly on the IHCs, second, the solitary stereocilia were longer and fatter than normal, third, the large sterocilia were typically bent towards the OHCs These observations match the descriptions from the electron-microscopic studies It is important to note that clumping and bending of the stereocilia are not among the typical changes observed in poorly fixed specimens

In regions of the cochlea where a full complement of hair cells remained, all supporting structures of the organ of Corti were typically present and erect Even in regions where some hair cells had been destroyed, the supporting structures frequently appeared almost normal (Fig 27, panel B) In regions where only IHCs remained, it was possible to see loss of Dieter's cells and/or outer pillar cells, with partial collapse of the tunnel of Corti In certain regions of some traumatized ears, the organ of Corti had completely disappeared, and only a layer of low cuboidal cells lined the surface of the basilar membrane (Fig 27, panel D) Loss of the organ of Corti was typically accompanied by severe depletion of the layer of tympanic mesothelial cells that lie under the basilar membrane No changes were noted in the basilar membrane itself

In ears exposed to the highest sound levels, structural abnormalities were not always restricted to the organ of Corti In some ears there was acellularity of the limbus, usually in the apical half of the cochlea (Fig 27, panel

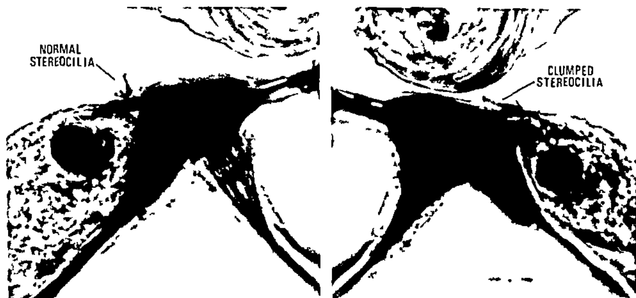


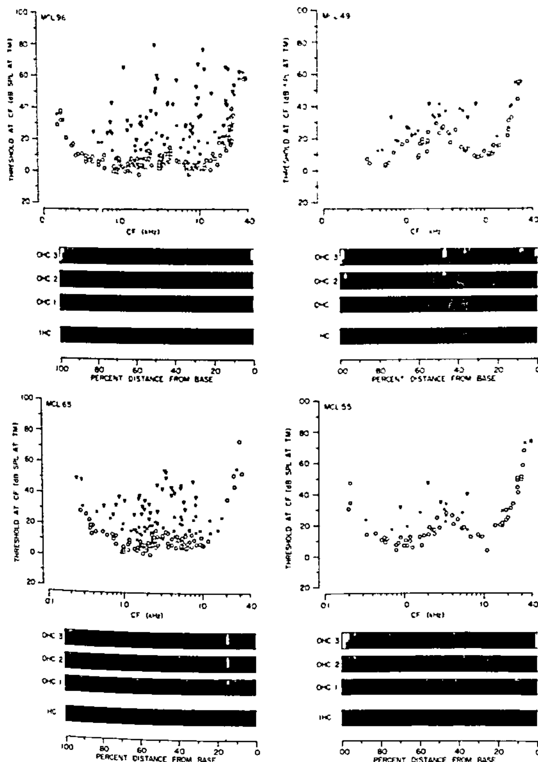
Fig 29 Photomicrographs illustrating the appearance of clumped stereocilia in a cross section through the organ of Corti The IHC with the abnormal stereocilia *(right panel) came from the upper basal turn of MCL* 71 the abnormal animal with the longest post exposure survival time (273 days) The normal appearing cell in the left panel was from the contralateral ear of the same *animal*

D) This degeneration was always in the region which Iurato (1967) has called the "central zone" The interdental cells of the surface zone were typically present even when the central zone was completely acellular, although in some cases the interdental cells showed some degeneration as well In some cochleas, there was acellularity in the spiral prominence (Fig 27, panel C) Again, this *acellularity was not seen in the surface epithelium* but in the deeper stroma of the spiral prominence Degeneration of the spiral ligament was seen in two ears, in the region near the spiral prominence in the basal turn Atrophy of the stria vascularis was seen only in one ear It should be emphasized that all of these pathological conditions were seen only in those ears with severe and extensive hair cell lesions In such ears, the damage to these structures was typically seen only in cochlear regions where the entire organ of Corti was destroyed The one striking exception to this rule was the degeneration of the spiral prominence, which was typically seen flanking a region of the cochlea where the organ of Corti had been completely destroyed In the results that follow, the reader should assume, unless otherwise specified, that for any cochlear regions where the hair cell destruction was subtotal, the histopathological signs were confined to the organ of Corti

B. Damage to the primary neurons

Degeneration of afferent nerve fibers seemed *to follow the pattern of IHC loss, significant* degeneration was never seen in cochlear regions where the IHCs were intact, even if the OHCs were destroyed As early as two weeks after exposure (the earliest survival time investigated), degeneration of the most peripheral portion of the afferent neurons was evident in the habenular regions radial to the areas of IHC loss With greater survival time, the peripheral processes of these neurons appeared to degenerate progressively towards the cell bodies As early as 24 days after exposure, large portions of the cochlea showed an osseous spiral lamina almost totally devoid of nerve fibers (Fig 27, panel D)

Loss of spiral ganglion cells was not evident in any of the ears with survival times less



ig 30 Comparison of cytocochleograms and CF hreshold plots for four normal ears In each cytococh eogram the percentage of cells remaining in each 1% f cochlear length is plotted in black for each of the four ows of sensory cells The distance dimension in the ytocochleograms is aligned with the CF scale in a simple linear-distance to logarithmic frequency relation The apex and base of the cochlea are aligned with 0 16 and 52 kHz, respectively, so that the correspondence between CF and cochlear location is that suggested by Schuknecht s data (1953)

than 45 days In all ears with survival times greater than 84 days, there was significant loss of spiral ganglion cells wherever there was significant loss of IHCs In one animal (MCL 71), with a large stretch of missing IHCs in the basal turn and a survival time of close to a year, there was nearly complete loss of spiral ganglion cells over a large part of the basal turn

III CORRELATIONS BETWEEN HISTOPATHOLOGY AND PATHOPHYSIOLOGY

A. Physiological deficits associated with hair cell loss

In recent years, the histological measure which has been used most commonly when comparing a functional deficit with inner ear damage is the "cytocochleogram' The cytocochleograms in this paper (Figs 30–33 and 35–40) plot, in black the percentage of hair cells remaining as a function of distance along the cochlear partition The crosshatching denotes regions of hair cell damage which will be discussed in part B of this section of the results

The cytocochleogram is frequently displayed so that its horizontal axis (distance along the cochlea) is aligned in some fashion with a frequency axis, along which is plotted some measure of auditory function In Figs 30–33, the cochlear distance dimension has been aligned with the CF dimension of single unit threshold plots Such an alignment implies the existence of a cochlear length frequency map i e , an algorithm assigning a CF to each point along the cochlear partition For the cat, the only published cochlear map is that of Schuknecht (1953), which was obtained by correlating behavioral hearing losses with cochlear lesions The equation representing the best straight line fit to Schuknecht s data has been used in correlating histological and physiological data in Figs 30–33

Shown in Fig 30 are data from four cases in which the thresholds at CF were within the normal range One case was a chamber raised animal (MCL 96), one was a control (MCL 49) and two were exposed to narrow band noise (but still showed normal thresholds) The hair cell losses in these four cases are minimal except at the extreme basal and apical ends of the cochlea Note, however, that the ear with the highest thresholds in the 3 kHz region showed what may be a significant loss of OHCs in roughly the middle of the cochlea

The cytocochleograms for four abnormal ears are shown in Fig 31 For the two cases in the bottom row, the threshold shift approaches 60 dB in some mid CF regions still, there is only a sprinkling of hair cell losses in any cochlear region outside of the basal and apical ends The two cases in the top row showed less shift in CF threshold, however, both ears showed slightly greater OHC losses in the mid cochlear regions These losses appear significant in comparison with those in the normal ears (Fig 30) One possible explanation for this discrepancy could be the difference in survival time, MCL 66 and MCL 77 survived for 57 and 58 days after the noise exposure, while MCL 64 and MCL 62 were sacrificed after only 28 and 15 days (Table I) The appearance of scattered OHC loss may require survival times longer than one month

In three of these abnormal ears a clearly significant loss of hair cells was seen in the most basal region of the cochlea known as the hook' (Note that these lesions are larger in the two ears with longer survival times) In each case, the hook lesion was unilateral, suggesting that it was created by the experimental noise exposure However, it seems most unlikely that these hook lesions were responsible for the threshold shift in the mid frequency region

Three cases with punctate, mid cochlear, hair cell lesions are shown in Fig 32 Each of these ears was exposed to narrow band noise centered at 6 kHz In each case there was a region of cochlea (roughly 40% of the distance from the base) where all of the hair

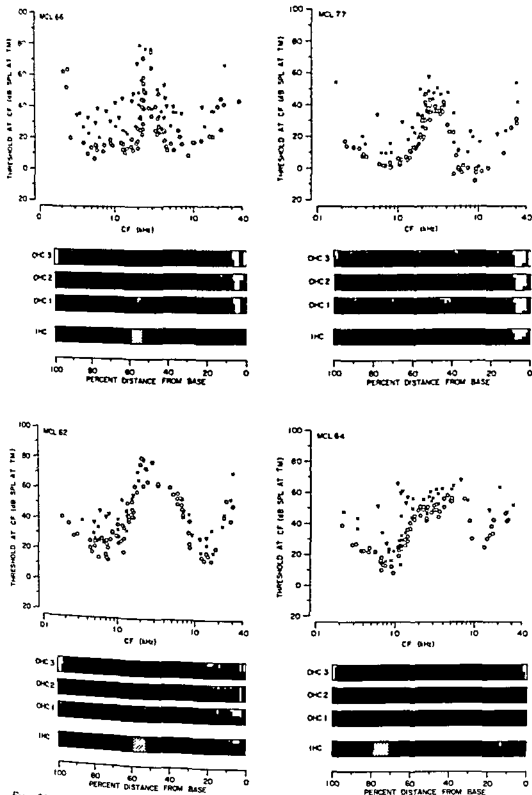


Fig 31 Comparison of cytocochleograms and CF threshold plots for four abnormal ears. Cochlear regions denoted by crosshatching in this and subsequent figures represent those regions in which clumping of the stereocilia was seen on the majority of the IHCs. The analysis of hair cell condition in those four ears was performed as part of the quasi blind analysis described in Methods.

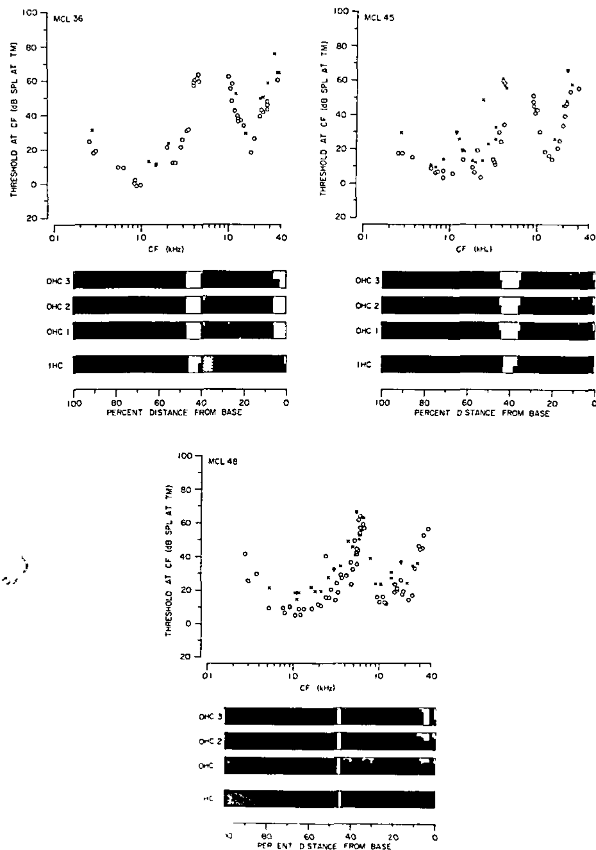


Fig. 32. Correlation between cytocochleograms and CF threshold plots in three animals with punctate regions of hair cell loss. Conventions for data display are as described for Fig. 31. In these animals the analysis of stereocilia condition was not performed as part of the quasi blind evaluation.

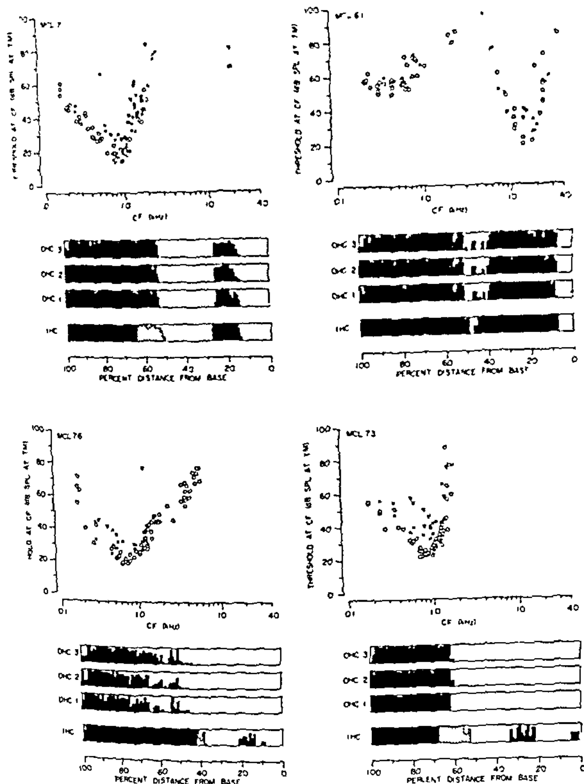
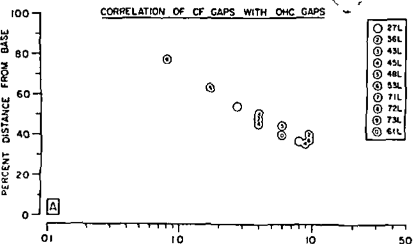
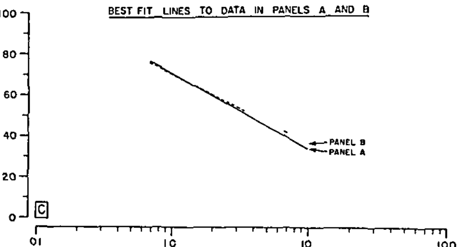
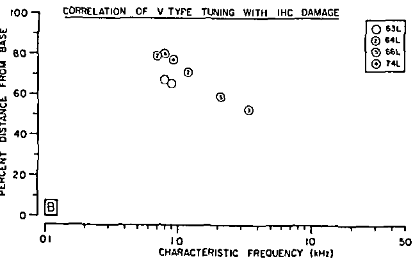
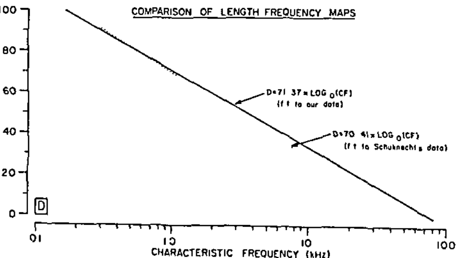


Fig 33. Correlation between cytocochleograms and CF-threshold plots in four animals with extensive hair cell lesions. Data are displayed in the same way as previously described. Evaluation of the stereocilia condition was not performed as part of the quasi blind analysis. The stereocilia in MCL 61 could not be evaluated because of poor fixation in that ear. In MCL 76 the spiral prominence was acellular in the cochlear region extending from 42–48% of the distance from the base.


CORRELATION OF CF GAPS WITH OHC GAPS
A
PERCENT DISTANCE FROM BASE
27L
36L
43L
45L
48L
53L
71L
72L
73L
61L


BEST FIT LINES TO DATA IN PANELS A AND B
C
PANEL B
PANEL A


CORRELATION OF V TYPE TUNING WITH IHC DAMAGE
B
PERCENT DISTANCE FROM BASE
CHARACTERISTIC FREQUENCY (kHz)
63L
64L
66L
74L


COMPARISON OF LENGTH FREQUENCY MAPS
D
(fit to our data)
(fit to Schuknecht's data)
CHARACTERISTIC FREQUENCY (kHz)

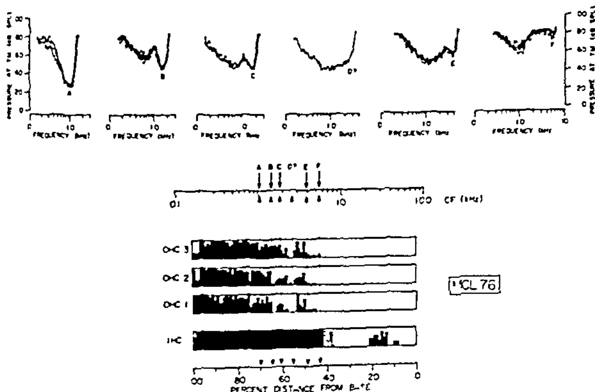


Fig 35 Tuning curves from six CF regions in one abnormal ear with extensive OHC degeneration Each of the panels in the upper row contains the tuning curve(s) of one or more high SR units from this animal The letter in each panel is positioned at the CF The CF dimension (as represented by the frequency scale in the middle of the figure) has been correlated with the cytocochleograms (by means of the dotted lines and arrows) according to the cochlear length frequency map suggested by the data from panel A of Fig 34

Fig 34 Comparison of various suggested length-frequency maps for the cochlea *Panel A* Each point in this plot is placed by correlating the border of an abrupt OHC lesion with the frequency at the border of the CF gap in that ear Correlations were attempted only if the unit sample was adequate to define the gap closely The key in the upper right allows identification of the case from which a particular point (or set of points) arises The cochleograms and CF threshold plots for several of these cases can be found in Figs 32 and 33 *Panel B* Each pair of numbered points in this plot is placed by correlating the borders of a cochlear region in which the IHC stereocilia were clumped with the borders of the CF region from that ear in which the tuning curves showed the type V abnormality The cochleograms and CF threshold plots for three of these cases are shown in Fig 31 *Panel C* A comparison of the best fit straight lines to the data in panels A and B Best fit is determined by the method of least squares *Panel D* A comparison of the cochlear length frequency maps suggested by our data including points from both panels A and B (solid line) and by Schuknecht s data Best fits are determined by the method of least squares The equations shown for the two maps are expressed in such a way that a CF value in kHz will yield a cochlear location in terms of percent distance from the base

cells, both inner and outer, were destroyed In the unit threshold plot for each ear there seems to be a gap in the distribution of CFs, the size of the gap being significantly smaller in the ear with the most restricted lesion (MCL 48) One notices, however, that the hair cell gaps and the CF gaps do not "line up" according to Schuknecht s length frequency map for the cochlea It seems reasonable to assume that the gaps in the CF distribution were directly attributable to the punctate lesions of the organ of Corti and, thus, that Schuknecht's map is not sufficiently accurate in the present context

The data from Fig 33 provide some evidence that the location and extent of such CF gaps are better correlated with the OHC lesion than with the IHC lesion Compare for example the data from MCL 71 and MCL 73 In both ears the border of the IHC lesion

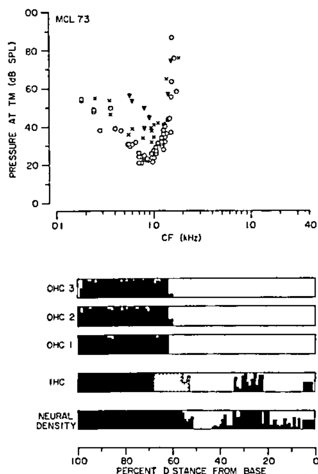


Fig 36 Pattern of peripheral degeneration of auditory nerve fibers compared to the pattern of hair cell loss and abnormal thresholds in one acoustically traumatized ear The neural density plotted in the bottom histogram represents an estimate of the proportion of nerve fibers remaining in the osseous spiral lamina in the vicinity of the habenula perforata There was no detectable loss of spiral ganglion cells in this ear

occurs at just over 50% of the distance from the base The border of the OHC lesion occurs more apically in MCL 73 and correspondingly the CF gap in that ear extends to a lower frequency Furthermore only in MCL 71 are there OHCs in the basal turn and only in MCL 71 were there any units with sharp tuning curve tips at the high frequencies

If the presence of a CF gap is in fact due to a total regional loss of OHCs the cochlear length frequency map that would be derived from such a correlation is shown in panel A of Fig 34 Data are included only from those ears in which the borders of the OHC lesion were abrupt The data conform reasonably well to a linear distance to log frequency relation

If we accept for the moment the cochlear length frequency map suggested by the best fit line to the data in panel A of Fig 34 we can examine the correlation between physiology and histopathology in an ear in which the borders of the outer hair cell lesions are not so abrupt MCL 76 is a particularly interesting example As shown in Fig 33, the unit sample from this ear showed a clear CF gap for frequencies above 5–6 kHz and what may be a smaller CF gap centered near 3 kHz As shown in Fig 35 the border of the CF gap at 5–6 kHz is correlated with the most basal location where OHCs are present Interestingly, the smaller CF gap near 3 kHz is associated with a small island of complete OHC destruction, bounded apically and basally by regions with significantly more OHCs As illustrated in panel D of Fig 35, there were in this ear, units with tuning curves so broad that no CFs were assigned It is possible that such tuning curves represent the response of fibers innervating the IHCs in the region where the OHCs had been selectively destroyed In fact, if the array of tuning curves obtained from this animal is arranged according to the highest frequency on the tuning curve, the bowl shaped curve from panel D does fall between those in panel C and those in panel E

If the loss of the sharp tip of the tuning curve were due to the loss of OHCs, there should be some correspondence between the degree of elevation on the tuning curve tip and the amount of subtotal OHC loss Some evidence for this correlation can be found in the data from MCL 76 where there was a large cochlear region with selective destruction of significant numbers of OHCs This gradual decrease in OHC population as a function of distance from the base was correlated with an exceptionally gradual increase in the unit thresholds for CF above 700 Hz Additional evidence can be found in Fig 33 Note that

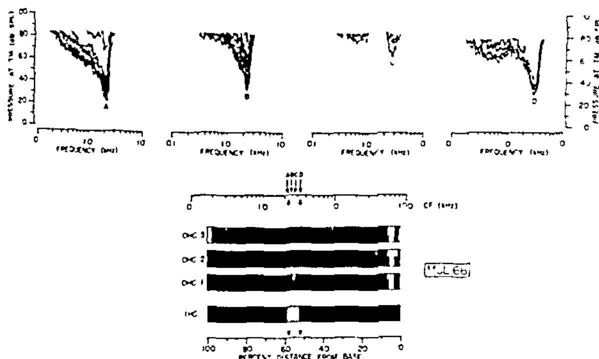


Fig 37 Comparison of tuning-curve shape and the patterns of hair cell loss and damage in one acoustically traumatized ear (MCL 66) The four panels in the top row display all the tuning curves ($n=33$) obtained from units with CF between 2 1 and 3 2 kHz The conventions for data display are as described in captions to Figs 31 and 35

in each of these cases there were no undamaged units in any CF region, i e , even the most sensitive units were more than 20 dB from the BTC Associated with these damaged CF regions was significantly more scattered loss of OHCs than in any of the normal ears Thus, although elevation of tuning curve tips was not always clearly associated with loss of OHCs when significant OHC loss could be discerned, it could be correlated with the elevation of threshold at CF

Thus far, attention has been focused on the physiological correlates of OHC loss What about the possible consequences of IHC loss? Significant IHC loss was invariably associated with massive or total OHC loss In all ears in which there was extensive loss of IHC there was also extensive degeneration of the peripheral processes of the auditory-nerve fibers The overall pattern of this degeneration was similar to the pattern of IHC loss, as shown in Fig 36 When the survival time was less than several months, the full complement of spiral ganglion cells remained, with apparently normal central processes When microelectrode penetrations were made through the auditory nerve in an ear with extensive degeneration of the peripheral processes of the neurons, the density of single units seemed normal (if electric shocks were used as the search stimuli) The normal unit density in MCL 73 (shown in Fig 21) suggests that neurons with degenerated peripheral processes (Fig 36) can still be electrically excitable and are capable of conducting impulses These abnormal neurons may correspond to the population of single units which show no SR and do not respond to sound In MCL 73, the location of these "silent" units suggested that the majority originally had CFs in the medium- and high-frequency range (Fig 14) This deduced CF range is consistent with the distribution of the partially degenerated fibers in that ear (Fig 36)

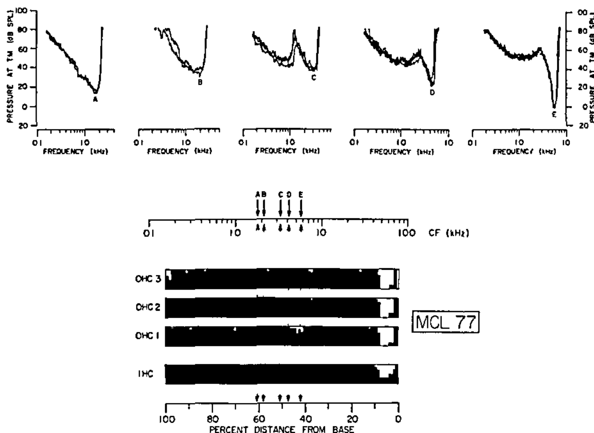


Fig 38 Selected tuning curves for five CF regions in one abnormal ear without clumping of IHC stereocilia All conventions for displaying data are as described for Figs 31 and 35

B. Physiological deficits associated hair cell damage

above comparisons have shown that the conventional cytocochleogram is limited as a predictor of pathophysiology While it is true that the existence of a cochlear region with total loss of OHCs could always be associated with a gap in the distribution of assignable CF, there were many cases in which no significant hair cell loss could be associated with significant abnormalities in single-unit thresholds

The conventional cytocochleogram displays only the pattern of hair cell loss and does not imply that hair cells scored as "present" are either structurally or functionally intact In making the hair cell counts in normal and abnormal ears, we found that differences could be recognized in the condition of the cytoplasm, nucleus, and stereocilia of the remaining sensory cells The most prominent features of the differences were discussed above and illustrated in Figs 28 and 29 These histologic features of hair cell condition were evaluated in all those ears that showed only minimal hair cell losses At the time, only 17 of these ears had been processed histologically, of those 17, three had not been well perfused The remaining 14 ears (7 normal and 7 abnormal ears) were evaluated in quasi-blind fashion (see Methods)

There was no clear correlation between the pattern of hair cell pyknosis/crenation (Fig 28) and the pattern of unit-threshold shift In almost all ears, the IHCs showed an increasing gradient of pyknosis from apex to base, regardless of the pattern of thresholds at CF Although there were very few examples of pyknotic OHCs in the two ears with the best unit thresholds (of the 14 ears analyzed), the degree and the location of the OHC pyknosis and crenation in the abnormal ears was poorly

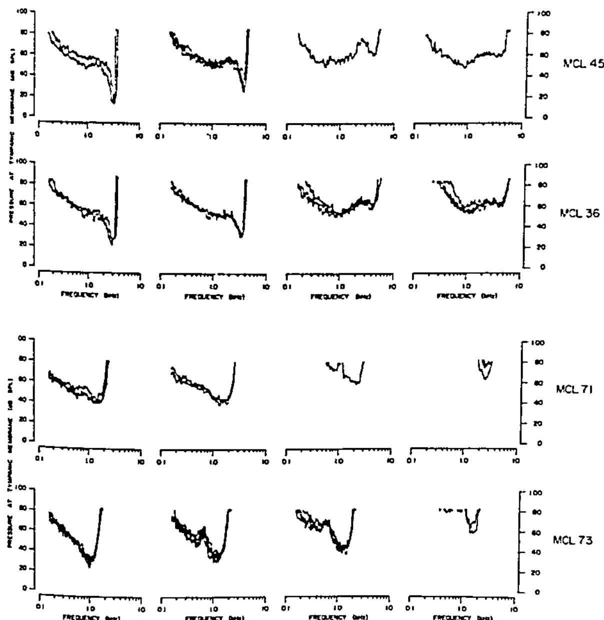


Fig 39 Tuning curves for units with CF at and below the CF gap in each of four abnormal cases Each row contains data from a different animal The extreme right hand panel of each row contains the tuning curves of the units with CF just at the low frequency border of the CF gap Each of the two ears represented in the lower two rows exhibited extensive clumping of the IHC stereocilia in cochlear regions on the apical border of the hair cell gap (see Fig 33) As can be seen from Fig 32 the two ears represented in the upper two rows did not

correlated with the degree and pattern of shift in unit thresholds It may be that variations in the quality of fixation had introduced differences in cellular characteristics that were superimposed on any pre-mortem differences in cellular outline and/or stainability

In the analysis of stereocilia condition, abnormalities were only noted on the IHCs Clumping or disappearance of the IHC stereocilia was almost entirely restricted to 4 exposed ears, each of which showed abnormal electrophysiology In these 4 ears, the abnormalities in stereocilia were found within a cochlear region less than 2 mm in length

Within that region, virtually all the IHCs (as seen in every fifth section) showed clumping of the stereocilia In the 3 other abnormal ears, there did not appear to be any such abnormalities. In the 7 normal ears, stereocilia clumping was noted in 3 cases, but only within the apical 4% of the cochlear duct

Data from 3 of the cases with extensive clumping of the IHC stereocilia were shown in Fig 31 Note that the cochlear locations of the damaged stereocilia in MCL 62 and MCL 66 (denoted by crosshatching) were almost identical and that both ears showed maximal elevation of unit thresholds for units with CF near 3 kHz (Both ears had been exposed to a noise-band centered at 3 kHz) The remaining 2 cases in Fig 31 serve to illustrate that in other abnormal ears the condition of the IHC stereocilia was not always correlated with the shifts in CF thresholds MCL 77, for example, was also exposed to narrow-band noise centered at 3 kHz In this ear there was a threshold shift of up to 40 dB, but no stereocilia abnormalities were seen In MCL 64, there was a restricted region of the cochlea where the stereocilia were rated as clumped, but the longitudinal extent of this region does seem comparable to the extent (in the CF on) of the shifts in threshold

Although there seemed to be no strict relation between abnormal stereocilia and threshold shift at CF, there was a tight correspondence between the clumping of stereocilia on the IHCs and the presence of type-V tuning curves In the physiological data from each of the 4 ears with stereocilia abnormalities, there was a distinct CF region populated exclusively by type-V units In the other 10 ears (for which there were no regions of clumped stereocilia), there were no CF regions in which medium- and high SR units showed the type-V abnormality

One example will serve to illustrate this correlation MCL 66 (Fig 37) was exposed to the 3-kHz noise-band and had a mid cochlear region of approximately 1 5 mm in length in which virtually all the IHCs showed clumped stereocilia (All the intermediate sections in this 1 5 mm region were stained and evaluated) The four panels in the top row of Fig 37 contain the tuning curves of all the units obtained in this ear with CFs between 2 1 and 3 2 kHz Only in this CF region did the tuning curves have hyposensitive tips and tails The tuning-curve shapes changed substantially for units with CF below 2 1 kHz and above 3 2 kHz (Fig 24) The cochlear location associated with these type-V tuning curves (according to the cochlear map suggested by the data points in panel A of Fig 34) is indicated by the dotted lines extending from the cytocochleogram to the frequency scale in the middle of the figure These type-V tuning curves appear to be correlated with clumping of the IHC stereocilia

Type-V tuning curves were not seen in any of the ears that did not show regions of clumped stereocilia Consider the example of MCL 77 (Fig 38) This ear had been exposed to narrow-band noise centered at 3 kHz and showed a significant shift in unit threshold The IHCs appeared normal in the light microscope, and the tuning curves in the 3-kHz CF region (panel C) were type-W

A summary of the correlations between type-V tuning and abnormalities of the IHC stereocilia (in those ears with minimal hair cell loss) is shown in Fig 34 (panel B) The best-fit line to these data is compared (in panel C) to the best-fit straight line to the data correlating CF gaps and OHC gaps

There also seemed to be a close correspondence between type-V tuning and IHC stereocilia abnormalities in those ears with significant hair cell loss In these ears, clumping of the IHC stereocilia was seen only at the borders of the hair cell lesion When stereocilia were clumped, the units at the borders of the CF gaps showed hyposensitive tips and tails (Fig 39, bottom two rows) When the stereocilia appeared normal at the borders of the hair cell gaps, the units at the border of the CF gap had tuning curves with hyposensitive tips and hypersensitive tails (Fig 39,

top two rows) Another example was shown in Fig 35 The IHC stereocilia appeared normal throughout the entire region where the OHC destruction was subtotal This was correlated with a large CF region in which the units showed type W tuning curves many of which had tip thresholds higher than tail thresholds

In all the data from ears with hair cell lesions the clumping of IHC stereocilia in a cochlear region where the OHC destruction was subtotal could be correlated with a CF region where tuning curves showed hyposensitive tips and tails The converse was not always true in one ear there was a CF region showing the type V abnormality which could not be correlated with abnormalities of the IHC stereocilia at the level of resolution offered by light microscopy

Discussion

This study began as an attempt to change the operational characteristics of the cochlea by modifying its structure in definable ways It was hoped that the structural changes following stimulation by high-intensity sounds would be reflected in reproducible physiological effects In most experiments on acoustic trauma there are many uncontrolled variables, and the present study is no exception Acoustic trauma, as a term, identifies only the stimulus producing the damage, not the nature of the damage itself The types of tissue damage may differ significantly, depending on the intensity range and/or the spectral composition of the stimulus Quite likely the specific locus and extent of damage will depend not only upon the characteristics of the sound but also on the susceptibility of the individual subject

A problem for any study of acoustic trauma which samples the functional state at a single time is the lack of assurance that the lesion is stable By waiting at least a month after exposure, we had hoped to be past the most acute stages so that functional changes would be *more likely to have a structural basis that* could be seen in light microscopy (Miller, Watson & Covell, 1963) There were hints in the data that at least some of the light microscope manifestation of the damage may still be slowly changing at survival times of a few months Certainly, the neuronal pathologies in spiral ganglion cells appear to be best interpreted as having a time course on the order of months Thus, it must be emphasized that the correlations we have presented between histopathology and pathophysiology can only be said to hold for survival times on the order of one to three months

Relation of traumatizing stimulus to the patterns of damage

Although this study was not designed to explore systematically the relationship between the traumatizing stimuli and either physiological or anatomical defects, a few pertinent observations can be made There seemed to be at least two separate foci for noise-induced damage to sensory cells One appeared in a cochlear region that could reasonably be associated with the spectral peak of the acoustic stimulus A second was often found at cochlear locations near the extreme base that correspond to frequencies about 30 kHz even though the traumatizing stimulus contained virtually no energy at such high frequencies (Figs 31 and 32) These lesions in the "hook" region of the cochlea were characterized by losses of OHCs and sometimes IHCs as well In some cases, they were more extensive than *the lesions associated with the dominant frequency* components of the traumatizing stimulus At sound levels that produced minimal lesions, these separate foci were separated by a large cochlear region with normal numbers of sensory cells For cochleas showing more severe damage, the lesions seemed to spread from the foci until they coalesced (Fig 33) Since the hook lesions were not found in the contralateral (unexposed) ears, these hair cell losses were almost certainly a direct result of the experimental noise exposure and not the

consequence of other factors such as environmental noise, ototoxic agents, congenital defects, or aging A similar pattern of damage has been observed bilaterally in monkeys after binaural stimulation (Moody et al , 1976) With bilateral lesions, of course, it is less certain that the damage was not a preexisting one

It is a matter of controversy whether there is another special focus of noise induced damage, the cochlear region that corresponds to frequencies near 3–4 kHz (Kellerhals, 1972) It was possible in this study to obtain physiological and anatomical defects that did not extend to the 3–4 kHz region by using 200-cycle bands of noise centered at 6 kHz (Fig 32) Furthermore, there do not appear to be any especially severe losses when 100-cycle bands of noise centered at 3 kHz were used (Fig 31) Thus, the present data do not support the idea of a special weakness in the 3–4 kHz region for narrow-band stimuli

There are, however, indications that there is something unique about the 3–4 kHz region in the cat Many units in the 3–4 kHz region of both exposed and unexposed animals show relatively high tip thresholds and relatively low tail thresholds, there are even some signs of a "3 kHz notch" for chamber-raised animals (Liberman, 1978) Whether the high thresholds for this CF region arise from a different kind of acoustic trauma (perhaps from broader band stimuli), accumulated, long term noise exposures temporary threshold shifts resulting from the preparations for recording inherited factors such as vascular insufficiencies, or from the interactions of these variables is unknown That the 3 kHz notch should be considered a pathological condition (though it is seen in nearly every case) is suggested by the fact that it is less obvious in chamber raised animals

Cochlear length-frequency maps

The correlation between pathophysiology and histopathology in noise exposed ears, as summarized in Fig 34, suggests a log frequency to linear-distance relation for the cochlear partition A direct test of the length frequency map for the cat would be to inject neurons of known CF with material that could be traced to the endings under the hair cells Unfortunately, this has not been done, so that, at present, the only test of the length frequency map suggested here is in the degree of accuracy with which it allows us to predict the overall pattern of histopathology from the pathophysiology One such test is shown in Fig 40 This case was sacrificed more than a year after exposure to high level noise The data were gathered long after all the other data in this report were analyzed and as this paper was being written Using our cochlear length frequency map, one would have predicted the presence of type-V tuning curves in the CF region below 1 7 kHz (panels A and B), since there were abnormalities in the IHC stereocilia in the cochlear region associated with these CFs In the CF region around 16–18 kHz, one would have predicted an elevation of the tuning curve tip and hypersensitivity of the tuning-curve tail (panel F), since there was a selective destruction of OHCs

As previously mentioned, the cochlear map suggested by the present data differs somewhat from Schuknecht's map derived from data correlating behavioral hearing losses and location of cochlear damage (Schuknecht 1953) It may be that the frequency region of maximal behavioral hearing losses is not the same in a given ear as the CF region showing maximal threshold shifts in auditory nerve units However a somewhat simpler explanation is that the discrepancy arises because of the relatively crude frequency resolution (octave intervals) of the behavioral data and the lack of sharp boundaries for the lesions In a more recent report of the correlation between behavioral hearing losses and noise induced cochlear lesions in the cat (Dolan et al 1975) one animal had a very restricted mid cochlear lesion following exposure to a 4 kHz tone The location of the lesion was roughly midw between the apex and the base The m

Discussion

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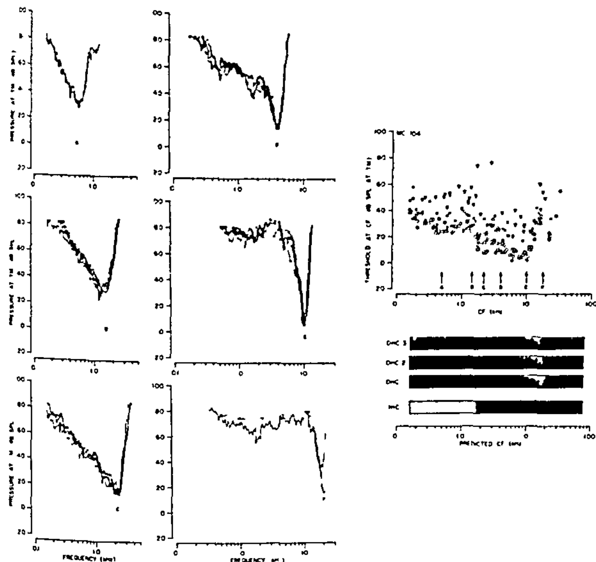


Fig 40 Comparison of physiology and histology in a noise-exposed ear sacrificed subsequent to the formulation of the cochlear length frequency map from Fig 34 This ear was exposed to narrow band noise centered at 750 Hz (bandwidth 50 Hz) at 112 dB SPL (rms) for two hours and allowed to survive for 18 months prior to the physiological experiment Tuning curves from six CF regions in MCL 104 are displayed in panels A through F (solid curves) The stippling superimposed in each set of curves encloses the tuning curves of all the high SR units in the appropriate CF region from a chamber raised animal The cytocochleogram (generated by the surface preparation technique) is aligned with respect to the CF threshold plot according to the cochlear length frequency map from panel D of Fig 34 Crosshatching indicates those cochlear regions in which there was significant damage to the stereocilia as evaluated by an observer with no knowledge of the physiological data

the tail (Wiederhold & Kiang, 1970) If, as reported by Iurato (1964), the crossed olivo cochlear bundle innervates exclusively, or even predominantly, the OHCs, then one might conclude that efferent activity influences the tips more than the tails of tuning curves because of its effect on OHCs How the OHCs could affect either IHCs or auditory nerve fibers still remains a matter of conjecture

One potential difficulty with associating tuning curve tips and OHCs is that tip thresholds were elevated in some cases in which the OHCs were present in normal numbers

As noted earlier these cases were most often those with the shortest survival times (less than 1 month) Some preliminary data gathered from noise-exposed animals with survival times greater than a year suggest that the disappearance of OHCs may continue for many months after the noise exposure Thus it may be that with longer survival times the damaged OHCs will disappear and significant elevation of tip thresholds will always be correlated with scattered OHC loss

Another phenomenon that may be related to OHC abnormality is the hypersensitivity of the tuning-curve tail associated with the elevation of tip thresholds (Kiang Liberman & Levine 1976 Schmiedt 1977) In any one ear hypersensitivity when present occurs only at certain frequencies and only for units in a limited CF range Thus the phenomenon must be intracochlear in origin since influences at the external or middle ear level would be seen in the tails of all the high CF units If tail hypersensitivity occurs because of selective damage to the OHCs (as suggested by the present interpretation of the tuning-curve arrays) one might conclude that the normal tuning curve reflects some non facilitatory teraction between IHCs and OHCs for fre ncies off the CF Perhaps the appearance hypersensitivity is related to the disappearance of two-tone inhibition which can occur with noise induced elevation of tip thresholds (Schmiedt 1977 Liberman unpublished observations)

Spontaneous activity and cochlear damage

Acoustic trauma can apparently affect spontaneous activity in auditory nerve fibers When there was a change in the pattern of discharge it could often be easily noticed since normal auditory nerve fibers do not exhibit "bursty" discharges It is more difficult to demonstrate a change in the average rate of discharge since the array of normal nerve fibers ordinarily exhibits a br d range SRs

In certain CF regions of traumatized animals there was a decrease in the range of rates as compared with that of chamber raised animals This compression of the SR distribution was associated with type V tuning curves which in turn appeared to be associated with damage to the IHCs Selective damage to the OHCs on the other hand was typically not accompanied by an overall depression of SRs as can be seen by examining the data from MCL 76 shown in Figs 25 and 35 Such observations are consistent with the notion that most spontaneous activity might be generated at the synapse between the IHCs and afferent fibers

Units unresponsive to sound had an even more severe compression of the SR distribution most had no spontaneous activity (Fig 19) These silent units were most often found in animals that showed extensive losses of IHCs and peripheral dendrites of primary neurons Thus the absence of spontaneous discharge may be associated with loss of the IHCs The few cases in which nonresponsive units showed spontaneous activity could correspond to fibers that innervated damaged IHCs at the border of a lesion The nonresponsive units that showed abnormal bursting patterns of spontaneous discharge might innervate IHCs in a different stage of the damage process In cats treated with ototoxic drugs the lack of acoustically nonresponsive units that retain spontaneous activity (Kiang Moxon & Levine 1970) might mean that in such ears there is a sharper border between normal and obliterated hair cell populations than exists in acoustically traumatized animals This interpretation is consistent with that suggested for the lack of type V tuning curves in those ears damaged by ototoxic drugs

Recently it has been suggested that in normal ears the population of auditory nerve units can be divided into three groups with different threshold properties according to rates of spontaneous discharge (Liberman 1978) It would be useful to know if the units

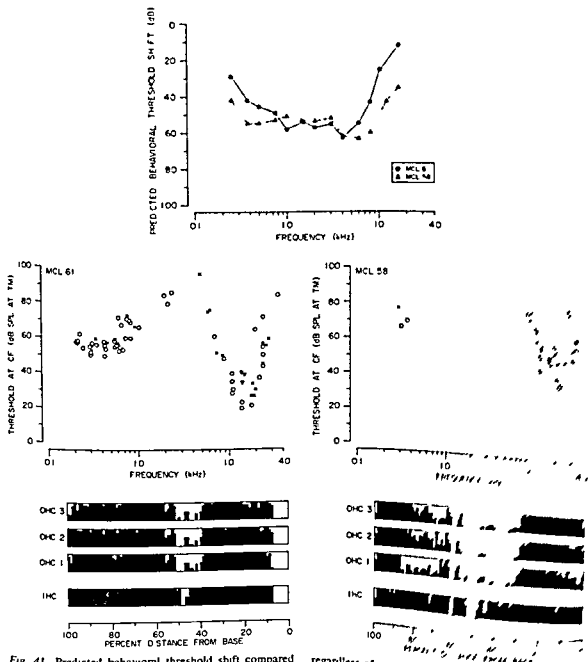


Fig. 41. Predicted behavioral threshold shift compared with CF threshold plots and cytocochleograms for two abnormal animals. The behavioral thresholds predicted for each case are estimated by determining the lowest single unit threshold found in each frequency range regardless of unit CF. [illegible] threshold (i.e. the [illegible] ference between [illegible] minimum threshold [illegible] normal ears.

in each of these three classes are similarly affected by acoustic trauma. It is difficult to answer this question conclusively so long as the possibility exists that acoustic trauma can alter the rate of spontaneous discharge in single units. [illegible] that a med[illegible] the same rate before the [illegible] level sounds [illegible] normal relation [illegible]

response to a given frequency, such changes might affect the appearance of behavioral masking functions such as "psychophysical tuning curves" (Zwicker, 1974, Vogten, 1974) These functions are obtained by determining the level required for a tone masker of variable frequency to mask the perception of a fixed tone just above threshold Since these masking functions in a normal subject resemble single-unit tuning curves with CF at the frequency of the fixed tone, it has been suggested that these functions map the composite tuning curves of the small group of units that have the lowest threshold to the fixed tone If true, the masking function run with a fixed tone near 4 kHz for an ear like MCL 61 would trace out the tuning curves of the 10 kHz units

Recently, this psychophysical technique has begun to be applied to humans with abnormal hearing, including individuals with noise-induced hearing loss What little of these data that has so far been reported is interesting in that abnormal psychophysical curves have been described which resemble the type-W abnormality seen in single units from acoustically traumatized cats (Leshowitz et al, 1976)

In the animal work, there are strict correlations between the pattern of abnormal tuning curve shapes and the pattern of cochlear pathology. If the human psychophysical data can be related to the single-unit work in animals, a clearer insight might be obtained into the cochlear pathology in humans with sensorineural hearing loss One could then correlate certain of the hitherto perplexing audiologic findings with knowledge of structural and physiological changes for various types of cochlear dysfunction

Summary of Results

A In animals exposed to high level narrow band noise the maximum shifts in thresholds at CF were often in the CF regions near the center frequency of the noise band When the threshold shifts were extensive the abnormalities always spread farther into CF regions above the spectral peak of the narrow band signal than to CF regions below (Figs 11 and 12)

B When the threshold at CF was elevated by acoustic trauma the shape of the tuning curve was frequently abnormal Of the units that retained some signs of sharp tuning two extreme forms of abnormal tuning curve shape were defined the type V shape for which the tuning curve tail and tip are hyposensitive the type W shape for which the tip is attenuated while the tail threshold is at least as low as (and can be lower than) that seen in normal units (Figs 5 and 8)

C The phenomenon of tail hypersensitivity was observed in some CF region of almost every noise exposed ear in which there were significant shifts in threshold at CF Hypersensitivity was observed regularly in units with high and medium SR and was most striking in units near the high frequency border of the abnormal CF region (Fig 15)

D Tuning curves with hyposensitive tails (and tips) were seen exclusively on the low frequency border of the abnormal CF region (Fig 15) The type V abnormality was present in both high and medium SR units (Fig 10)

E In any abnormal case the tuning curves for all units of similar CF and SR were similar in shape though not always in sensitivity (Figs 6 10 15 23 24 25 35 37 38 and 39) The tuning curves for high and medium SR units were almost always similar in shape in the same CF region of the same case (Fig 10)

F The normal relationship between SR and threshold at CF was often maintained in CF regions where the thresholds at CF were elevated (Figs 11 12 and 13) but there were exceptions (Figs 10 and 22) The degree to which the normal relationship was maintained appeared to be roughly correlated with the degree to which the normal distribution of SR was maintained

G The normal bimodal distribution of SRs was significantly altered in certain CF regions of some acoustically traumatized animals (Fig 19) This alteration was seen most clearly as an increase in the proportion of units with rates between 10 and 30 spikes/sec (Figs 23 and 24) This range of SR is only minimally represented in normal ears (Fig 16) The degree to which the SR distribution was altered was *not* strictly correlated with the degree of threshold shift at CF (Fig 22) except that those CF regions which retained normal CF thresholds typically retained a normal distribution of SRs (Fig 19)

H When the distribution of SRs showed an abrupt shift as a function of CF there was often an associated shift in the type of abnormal tuning curve shape (Figs 23 24 and 25) If the SR appeared to be depressed the predominant tuning curve abnormality was type V (Figs 23 and 24) In the one case when the distribution of SRs seemed shifted to

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VESTIBULAR DISTURBANCES AFTER ACUTE MILD HEAD INJURY

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VESTIBULAR DISTURBANCES AFTER ACUTE MILD HEAD INJURY

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I INTRODUCTION

Several sequelae such as headache, nausea, vertigo or dizziness, nervous strain, impaired ability to concentrate, memory loss or insomnia are frequently encountered after head injuries however slight they may have initially appeared to be These symptoms may invalidate the patient for several months Some authors feel that these symptoms, even when they are prolonged, always have an organic background, others, on the other hand emphasize the role of the psyche in the development of the postcommotional syndrome The clarification of the pathogenesis of the symptom is therefore of great socioeconomic importance with regard to the working ability and the rehabilitation of the patient

In spite of several investigations the pathogenesis of posttraumatic vertigo/dizziness has remained as yet unclear except in cases in which it is a result of a temporal bone fracture responsible for damages in the vestibular nerve or the inner ear Posttraumatic vertigo/dizziness is assumed to develop as a result of a damaged peripheral vestibular system, possibly due to otolith excitation Caloric tests and findings of spontaneous and positional nystagmus have corroborated this suggestion Unlike the cochlear system, the membraneous labyrinth does not display any specific histological changes after trauma

What happens in the brain at the time of injury is largely obscure It is difficult to know which traumas will be reversible (concussions) and which will be irreversible (contusions) if there is no massive hemorrhage Also the fundamental cause of traumatic unconsciousness is unknown is it primarily a disturbance in the cortex, subcortex or the brain stem, and is the disorder primarily vascular or neural? The reticular formation is considered to be of decisive importance in the pathogenesis of unconsciousness Combination of unconsciousness with central vestibular or cochlear disturbances could be explained by the role of brain stem injury in both mechanisms

II REWIEW OF THE LITERATURE

1 Peripheral pathophysiological disturbances

The pathophysiological mechanism of a head trauma in the vestibular region can be divided into direct and indirect effects Direct injuries to the ear may occur as results of fractures Transversal or longitudinal fractures of the temporal bone may run across the inner ear or the cochlear and vestibular nerves (in meatus acusticus internus) inflicting damages to these It is typical of these fractures that recovery is very slow or remains absent (Proctor et al 1956) Posttraumatic hemorrhages may be frequently detected histologically in the endo- and perilymphatic space (Schuknecht 1950 Proctor et al 1956) Blood cells may remain in the inner ear for a prolonged time without causing any hearing impairment (Proctor et al 1956 Schuknecht et al 1951, et al 1953 1969 Makishima & Snow 1976)

No specific histological changes have been observed in the peripheral labyrinth after the trauma (Schuknecht 1950 Makishima et Snow 1976) Secondary macula degenerations have been detected only after neuron lesions caused by severe head injuries and/or fractures (Schuknecht 1950 et al 1951 et al 1953 Proctor et al 1956 Makishima et Snow 1976) Henriksson (1974) suggests that otolith luxation might be a contributor to the vestibular disturbances present in head injuries

Several indirect pathophysiological mechanisms have been proposed Brunner (1925 1928) was the first to introduce the so-called vasomotoric theory (otitis interna vasomotorica) according to which disturbances in the microcirculation might be responsible for sensory cell injuries Axelsson & Halle (1973) were able to demonstrate that mechanic traumas are responsible for small microtrombs in the circulation of the inner ear Damages to central vasomotoric centres have also been found to result in disturbances in cochlear microcirculation (Makishima & Snow 1975) Wittmaack (1932) suggested that heavy intracranial hypertension might be conducted through the open aqueductus cochlea into the inner ear and thus inflict damage to sensory cells (commotio labyrinthi) Schuknecht (1950 et al 1953 1969) proposes on the basis of his investigations that a traumatic pressure wave may possibly be conducted to the inner ear through the cranial bones and cause an inner ear concussion which resembles a noise injury Several investigators regard a posttraumatic hearing loss as being pathogenetically identical to a noise injury (Ruedi & Furrer 1946 Escher 1948 Lehnhard 1974) Animal tests performed by Schuknecht (1950) prove that the cells of the upper basal coil (responsible for hearing at 4—8 kHz) are the most susceptible to injuries Next in order are the cells of the middle section and the apical cells are the last to be involved The outer sensory cells are more susceptible to damage than the inner cells (Schuknecht 1950 Ward 1969)

Ilberg (1977) maintains that the disturbance is combined and caused by vascular toxic and biochemical changes Disorders in the microcirculation may possibly be responsible for a local hypoxia which then results in the electrolytic changes in the endolympha

Electrolytic disturbances thus would inflict

damage to the sensory cells Boscher (1976) observed that oxygen deficit affects the permeability of the basillary membrane and thus the ion equilibrium of the endo- and perilympha High K^+ ion concentrations have been found to cause damage to sensory cells

2 *Central disturbances*

a Pathophysiology of brain injury

In spite of many detailed studies there is no absolute certainly of the primary dis turbance of consciousness after brain injury Strich (1961) suggests neural involvement to be primary and vascular metabolic and bio-chemical disorders to be secondary On the other hand Bruce (1976) and Rosenblum (1976) maintain that primary vascular dis turbances are important for the development of secondary brain tissue damage

— translation and rotation of head

It has been suggested that translation (i e movement in a straight line) of the head produces pressure gradients contributing signi ficantly to cerebral concussions as well as cerebral contusions (Gurdjian et al 1966 Unterharnschiedt & Higgins 1969 Walker 1973)

On the basis of his experimental data Ommaya (et al 1976) however suggests that the loss of consciousness and traumatic amnesias of common head injuries are due to diffuse bilateral damage to the brain and that no focal lesion can reproduce both of these aspects of traumatic unconsciousness He agrees that focal lesions (hemorrhages con tusions and lacerations) are key determinants for spesific neurological deficits — it is the diffuse set of lesions however which are the major determinants of the quality of the final outcome after head injury Ommaya (et al 1976) also agrees that sudden rotatory or angular movements of the head are necessary for loss of consciousness

Traumatic unconsciousness will not develop until the magnitude of shear strain is large enough to reach the well protected mesen cephalic part of the brain stem and thus complete the disconnection of the alerting system of the brain (Ommaya et al 1976) During the period of unconsciousness there is a lack of activity in the mesencephalic reticular formation (Ward 1969 Walker 1973) but the functional reconnection of reticular formation pacemaker activity and cortical function is difficult to determine (Jennett & Plum 1972)

Ponten (1973 et al 1974) assumed on the basis of his biochemical findings in cases of experimental head injury that the brain tissue itself is rather resistent to trauma but that secondary ischemic and hypoxic changes of complex pathophysiology and probably a very inhomogenous flow are responsible for much of the brain damage even in the acute phase

Makishima (et al 1976) found that occipital blows have the most intense effect on central disturbances after head injury Animals which received a temporal blow showed little or no signs of concussion

— intracranial pressure

The studies of the pathophysiology of the brain have concentrated on the mechanics of severe brain injury with increased intracranial pressure (Vapalahti 1970) and brain edema (Bruce et al 1973) There are few studies on the pathophysiology of brain concussion — mild injuries (Relander 1972) Animal models for this injury are extremely difficult and mostly unreliable for humans (Makishima & Snow 1976 Ommaya & Gennarelli 1976)

Troupp and Vapalahti (1967 1970 1971) showed that patients who died died with in creased intracranial pressure and that pressures over 60 mmHg almost always meant death in cases of brain edema This has been confirmed by Bruce (et al 1976) Becker (et al 1970) Overgaard (1976) andMiller (et al 1977) but

not by Tindall & Fleischer (1976) *In mild* cases there is no elevation of intracranial pressure Overgaard (1976) and Rapoport (1976) state that conventional methods for monitoring intracranial pressure are incapable of detecting critical pressure levels in the brain tissue

— cerebral blood flow and autoregulation

Radioactive isotopes and computerized axial tomography (CAT) have given an abundance of new information about the cerebral blood flow (CBF) *after head injury (Fieschi et al* 1972 Simeone et al 1972 Ommaya 1973 Lassen & Ingvar 1972 Ambrose & Hounsfield 1975 Rosenblum 1976, Obrist et al 1977) Taylor & Bell (1966) has shown that the mean circulation time in patients after head injury is slowed by 15 % Bruce (et al 1973) and Overgaard (1976) showed that in most patients the cerebral blood flow decreased even more although there are patients with increased cerebral blood flow *after severe brain injury A normal flow is not* an indication of normal brain function (Overgaard 1976)

The disturbances of autoregulation may also have an important role in the cerebral blood flow disturbances after severe head injury (Reivich et al 1969 Dila et al 1976 Enevoldsen & Jensen 1977) However this was not supported by the clinical material of Bruce (et al 1973) Dila (et al 1976) maintains that when the permeability of the capillaries increases *it may result local cerebral edema* (Reivich et al 1969 Rapoport 1976)

— neurotransmitters

The effects of histamin serotonin and prostaglandins on cerebral blood flow have been very intensely studied but their roles after head injury are not yet clear (Osterholm et al 1969 1974, Carter et al 1974 *DeLa Torre et* al 1974 Allen et al 1974 Adams et al 1976 *Rosenblum 1976) It has been found that serotonin metabolism increases during the time* of unconsciousness and so probably compensates for the disturbances (Hyyppa 1976, 1977) Most serotonin neurons are located in the brain stem region an area which can be affected by injuries (Hyyppa 1976 1977) With lumbal liquor determinations Vecht et al (1975) was able to demonstrate that the brain dopamin and serotonin metabolism are reduced after head trauma Great variations in levels of acetylcholine (Patrick & Pham *1978)* tryptophane (increased values) and 5 HIAA (decreased valueas) in all regions of the *brain have also been found after severe head* trauma (Vecht et al 1975, Hyyppa et al 1977, van Woerkom et al 1977)

b Histopathological findings

Experimentally induced trauma in animals demonstrated that even minor head injuries may produce petechial hemorrhage into the brain tissue particularly into the brain stem and vestibular nuclei It requires less force to *produce concussion in cats when the head* is free to move than when it is fixed (Denny—Brown & Russel 1941) Also Windle et al 1944) in well-controlled studies of the effects of concussive blows noted almost immediate pathological changes (chromatolysis) in the neurons of the brain stem and the spinal cord The vestibular nuclei were especially vulnerable in the brain stem They found no neuron destruction in the cerebrum the basal ganglia nor in the cortex Neither Denny—Brown nor Windle *examined the temporal* bones of their animals

Chason (et al 1956) states that pressure gradients at the craniospinal junction result in shear and tear of the elements in the brain stem Even subconcussive blows in dogs resulted in some cellular changes in the medulla the pons and the midbrain These *reversible* changes (subconcussive blows) may occur in *the cells of the brain stem and account for the* usual findings in the human case of concussion

Strich (1961 1969 1970) found widespread diffuse degeneration of the white matter in patients who died after head injury the lesion was caused by a large number of fat granule cells (phagocytic cells) The descending and ascending tracts of the brain stem were similarly affected In cases with short survival (up to 6 weeks) active myelin and nerve fibre degeneration was observed and there were a large number of nerve fibres with retraction balls (Voigt et al 1977) Microscopically the cerebral cortex appeared to be normal The changes are typical of the secondary or Wallerian degeneration which follows the interruption of nerve fibres from any causes Strich also says that the histological appearances are neither those of oedema which is characterized by the absence of fat and the presence of fibrous gliosis nor of anoxia which mainly affects the grey matter He concluded that the injury to the nervous tissue must be immediate and that oedema anoxia and vascular disturbances are secondary

Adams et al (1977) does not believe in the disruption of nerve fibres nor in total lesion of neuron groups as Strich (1961 1969 1970) does The stretching of nerve fibres evidently results in a transient impairment in the conduction of the nerve impulse These observations are supported by the results obtained by Oppenheimer (1968) He detected after an unconsciousness of a few minutes even in mild injuries formations of microglial stars which indicate a diffuse damage to the white matter (Adams et al 1977)

Nakamura (1967) and Bruce (1976) also found hemorrhagic lesions in the brain stem in their experimental studies and say that the impact distorts the brain within the skull and the stress caused by distortion then concentrates to the brain stem resulting in injury

Kirikae (et al 1969) found microscopically small areas of encephalomalasia in the temporal lobes In the brain stem the cochlear nuclei the superior olivary nucleus the lateral lemniscus nucleus and the inferior colliculus were degenerated by disseminated lesions hemorrhage oedema degeneration softening and necrosis

Varying incidences of hemorrhage in the primary brain stem have been observed by different authors Jellinger (1967) described hemorrhages in 43 5 % and Mayer (1967) in 100 % of their cases Budzilovitch (1976) found that hemorrhages in the brain stem were clearly related to the small arteries veins and/or capillaries while Mayer (1967) observed that the peripherally located hemorrhages were predominantly arterial and those around the aqueduct and the IV ventricle were venous in origin

Ommaya s (1966 et al 1974 et al 1976) histological findings after head injury in animals did not confirm the observations of other authors on consistent histological changes in the cervical cord and the brain stem structures He suggested that concussions are graded rather than being 'all-or none responses of the central nervous system Shear or tensile deformation within the brain and the cervical cord produced by sudden impacts or indirect acceleration of the head results in dysfunction of neural elements (Mayer 1967)

Makishima (et al 1976) studied histopathologically the brains and the temporal bones of patients who had died of head traumas and those of guinea pigs which had received a head blow with a pendulum device In human cases there were multiple areas of hemorrhage and necrosis in the cerebral hemispheres not only in the grey matter but also in the white matter Wide spread hemorrhages were prominent in the brain stem in most patients The cerebellum revealed an extensive loss of Purkinje cells Extravasation of blood in the internal auditory canal and the substance of the VIII cranial nerve in association with laceration of its fibres was found in each patient Red blood cells were found frequently in the scala tympani especially at the basal turn of the cochlea The membranous labyrinths demonstrated no specific changes secondary to trauma

If the cerebral cortex is histologically nor

mal it does not necessarily mean that there is no pathology because most neuropathologists agree that it is impossible to detect cellular losses until they have reached 35—50 %

In spite of many detailed pathological studies no completely adequate description of the relative three dimensional distribution of all primary lesions throughout the brain at known intervals of time after head injury is available in man or in experimental animals (Strich 1969 Unterharnscheidt et al 1969, Zulch 1969 Makishima et al 1976 Ommaya et al 1976) It is clear from the discrepancy between the marked physiological disturbance and often mild pathological evidence of damage in brain trauma cases that the pathological examination will have to be extended to deeper levels of analysis in order to achieve more precise clinicopathological correlations Ommaya & Gennarelli 1976) Most neuropathological studies come from fatal cases with large contusions and increased intracranial pressure There are no studies of the morphology of brain concussion

3 Electronystagmographic findings

— spontaneous and positional nystagmus

The mechanism of the frequent posttraumatic sequelae positional (PN) and/or spontaneous nystagmus (SN) has yet remained obscure in spite of several investigations (Barany 1906 Nylen 1950 Gordon 1954 Harrison 1956 Preber & Silfverskiold 1957 Lange & Kornhuber 1962 Barber 1964 Scherzer 1975) Otolith disturbances possibly otolith luxation have been proposed as being responsible for PN (Barany 1906 Schuknecht 1950 Henriksson 1974)

So-called benign positional nystagmus (BPN) accompanied by brief vertigo is frequent after head traumas (Dix & Hallpike 1952 Barber 1964 McClure et al 1977) In general BPN is found to occur with peripheral vestibular disturbance but Fernandez (et al 1959) reported BPN even in lesions of the cerebellum and the brain stem Some authors have suggested that BPN might arise from the excitation of utricular and saccular otoliths (Barany 1906 Schuknecht 1950, 1962 Barber 1964 Pearson & Borber 1973) Schuknecht (1962) found freely moving particles (cupulolithiasis) in the endolymphatic space which in his opinion may irritate the cupula Even blood cells may be responsible for cupular excitation (McClure et al 1973 1977) As a rule BPN patients display symmetrical caloric tests

There are several possible ways to classify positional nystagmus Nylen (1950) divided PN into three types This differentation is still practical provided the duration of nystagmus is taken into account Type I denotes positional nystagmus with directional changes at different head positions Type II the direction of nystagmus remain unchanged Type III the direction of nystagmus varies with alternating irregular phases of nystagmus I and nystagmus II Type III includes as a subgroup the so-called benign positional nystagmus (BPN) which is characterized by latent periods of a few seconds BPN can be provoked 3—5 times and for 30—60 seconds at a time which duration is seldom exceeded

Nylen (1950) maintains that posttraumatic PN of type I or type III indicates a central disturbance in the brain stem This might in turn contribute to the difference growing between central and peripheral tonus Also Lindsay (1951) regard the posttraumatic disturbance as being central

According to Harrison (1972) all PN types (I—III BPN) are equally often encountered after head trauma If a subject over 40 years of age reveals posttraumatic PN of type I Harrison regards this as a reflection of previous disturbance of vertebral circulation Also Aschan (1956) and Bergstedt (1962) observed that prolonged PN (over 60 seconds) indicated a central lesion particularly if there is no vertigo or if vertigo is slight

Harrison (1956) reported BPN in 18 % of his material with head injuries (104 patients) but a central lesion could be manifested in

only one of these Barber (1964) observed PN in 25 % of his head trauma patients, and nearly all of these were of type BPN Preber & Silfverskiold (1954), on the other hand, found a central ENG-disturbance in one third of his head trauma patients with verified BPN

Lange and Kornhuber (1962) examined 210 patients with contusions and 97 patients with commotions They observed SN in 11 % of both groups Posttraumatic SN persists several years and might even prove permanent (Fisch 1973) Podoshin & Fradis (1975) examined 395 patients with head traumas and 30 % (118) of these complained of vertigo PN of type 1 was established in 48 cases primarily but 6 months later in only 3 patients

Fluur (1974) has conducted thorough vestibular studies on test animals By selectively disrupting nerves of the utriculus, the sacculus and the ampulli, he observed that otoliths alone without any contribution of the end organ the semicular canals are not able to produce PN or SN Nor can PN or SN be provoked by destroying the sacculus (Miehlke 1955) or by irritating electrically the utriculus or the sacculus Fernandez (et al 1959) caused experimentally a cerebellar lesion and demonstrated the vital role played by the lack of central inhibition in the pathogenesis of PN or SN

PN and SN can be observed also in healthy persons if the recording is continued long enough, provided the recording apparatus is sufficiently sensitive (Vesterhauge & Larsen 1977) By using vertical and horizontal electrodes simultaneously, PN and/or SN can be found in as many as 80 % of healthy persons (Fluur & Eriksson 1961 Bos et al 1963) Jonkeens and Philipzoon (1964) set 6° and 7°/°/s as thresholds for pathological PN and SN, respectively 7°/s has frequently been regarded as the threshold for perceptible nystagmus with Frenzel s glasses In Vesterhauge's (et al 1977) study the threshold of pathological nystagmus was set at 6 5°/s Mulch & Lewitzki (1977) observed PN and/or SN in 60 % of normal persons when the threshold of 2°/s was employed

It may thus be concluded that on the basis of PN and/or SN only, the localization of the lesion cannot be determined with certainty if there are no temporal bone fractures (Eviatar et al 1968, et al 1971, Harrison et al 1972, 1975, McClure et al 1977)

— caloric tests

Results of caloric tests after head trauma vary greatly depending on the severity of the injury and the localization of the skull fracture (Perlman 1939, Proctor et al 1956, Kotova 1962 Lange & Kornhuber 1962, Davey 1965, Caveness 1966 Harrison & Ozsahinoglu 1972, Pearson & Barber 1973 Scherzer 1975) The incidence of canal paresis (CP) is high if the temporal bone is fractured (Proctor et al 1956) Directional preponderance (DP) may occur in peripheral, and central disturbances, even in healthy subjects which thus annihilates its diagnostic value in localizing the lesion (Decher & Sperling 1960, Parker & Weiss 1976)

Proctor (et al 1956) observed homolateral canal paresis in all patients with transversal temporal bone fracture If the fracture had affected other parts of the cranium, canal paresis occurred in 50 % of the patients, if no fractures could be detected, canal paresis could be established in only 28 % of the patients with head injury

Schader (1960) examined the ENG of 1451 head trauma patients 1—15 years after head injury, and found that 8 % of them had DP 17 % had CP, and only 7 % showed findings indicating a central disturbance Toglia (et al 1970) found CP in 69 % of 119 head injury patients

Pearson & Barber (1973) could not demonstrate any correlation between the severity of the injury and the results from caloric tests or changes in hearing Canal paresis could be homo- contra or bilateral to the impact In a follow up study Podoshin & Fradis 1975) found canal paresis immediately af

in 43 % of the patients (46/107) but 6 months later the proportion had dropped to 17 %

— pendeldeviation and gaze nystagmus

Horizontal pendular movements of the eye can often be found with fatigue even in healthy persons (Decher 1965 Scherzer 1975) Then the frequency of the pendular movement (1/3 Hz) corresponds to the cyclus of the formatio reticularis (Trincler 1966 Scherzer 1975) In patients with cerebral contusions Lange & Kornhuber (1962) found pendular movements in 33 % of the subjects and Scherzer (1975) in 24 % In milder injuries (concussions) the corresponding values were 14 % and 8 %

Lange & Kornhuber (1962) could establish gaze nystagmus in only 4 patients out of 297 Scherzer (1975) maintains that this nystagmus which is linked with central disturbances only occurs in the acute and the subacute phases after trauma

— rotational tests (nystagmus thresholds postrotatory nystagmus)

Decher (1965) and Montandon (et al 1962 1972) observed that nystagmus thresholds were elevated after head injuries and regarded peripheral vestibular disturbances as being responsible for this phenomenon They also recorded increased nystagmus frequencies frequent dysrhythmus wide-amplitudinal nystagmus and total prevention of nystagmus

Wilmot (1966) established ENG changes occurring immediately after trauma which nearly corresponded to those occurring with vertebrobasillary insufficiency and he suggests that these findings might refer to posttraumatic brain stem disturbances in the first place

Toglia (et al 1976) reported on pathological per and postrotatory test results in 32 % of the patients (48/152) Moser (1977) examined 270 patients with concussions who complained of vertigo/dizziness With rotational tests he established central disturbances in 31 % of the material

4 Posttraumatic hearing impairments

Posttraumatic pure tone audiograms frequently show lowered hearing capacity at 4 6 and 8 kHz (Proctor et al 1956 Lange & Kornhuber 1962 Schuknecht 1950 et al 1951 et al 1953 1969 Toglia et al 1970 Escher 1973 Lehnhard 1974) Hearing impairment is generally sensorineural The incidence rate varies between 30—70 % depending on the severity of the trauma (Proctor et al 1956 Rantanen et al 1967 Toglia et al 1970) As a rule hearing is improved within a few weeks but at high frequencies the recovery is slight (Hough 1969 Toglia et al 1970) The change in hearing may be contra or homo-lateral to the injury but normally it is found to be bilateral (Podoshin & Fradis 1975) Also fluctuations have been observed to occur in hearing as results of the pressure changes in the inner ear due to a disturbed flow in the cochlear aqueduct (Fee 1968 Stroud & Calcaterra 1970 Goodhill et al 1973) Severe gradual hearing losses have occasionally been established several months later due to secondary degeneration as suggested by Schuknecht (1969) Also impaired conductive hearing has been detected after head injuries particularly with fractures of the temporal bone (Tos 1971) Hough (1969) and Escher (1973) have obtained good operative results in treating posttraumatic hearing losses which have been caused by ossicular luxations or fractures

Fradis & Podoshin (1969) and Podoshin & Fradis (1975) have established in their investigations that blows to the occipital region cause the most severe hearing losses at 4 6 and 8 kHz Their results were in accordance with those of Makishima and Snow (1976) who studied the responses evoked potentials in the brain stem

On the basis of his results Lehnhard (1974) maintains that disturbances in the brain stem

can be observed after head traumas of relatively little impact energy Makishima and Snow (1975) measured posttraumatically the cochlear potentials of test animals and determined then the evoked responses in the brain stem In their well-controlled test they established normal cochlear potential values whereas the responses evoked in the brain stem were usually pathological which tends to suggest disturbances in this region The tests performed by Makishima & Snow (1976) on test animals have corroborated the assumption that central hearing impairment is frequent in head injuries His histological findings also confirm that blows to the occipital region are more perilous than those to the temporal region

5 EEG findings

One of the mildest effects of head injury is a local unilateral partial or complete suppression of the alpha rhythm (Franzen et al 1958) Partial suppression of the alpha rhythm may occur with apparently trivial injuries (Bickford & Klass 1966) In traumas of slightly greater severity alpha rhythm may reveal bilateral disturbances either with reduced frequency or with altered distribution alone or associated with the appearence of a few slow waves (Bickford & Klass 1966) The second grade of severity is reached when slow waves of 1—5 cycles/sec (cps) appear with varying amounts of synchrony Also 10—20 percent of normal subjects have pathological EEG (Bickford & Klass 1966)

Meyer—Mickeleit (1953) found of pathological EEGs in only 15 per cent of 606 patients with cerebral concussions No alpha rhythm suppression was found by Koufen (1977) in a material of children with head injuries

Some authors have found correlations between the severity of the trauma and the EEG findings (Beaussart & Beaussart Boulenge 1970 Lorenzoni 1970 Dichgans et al 1978) In whiplash injury Torres and Shapiro (1961) found a correlation in mild cases but not in severe traumas Steinmann (1967) and Gemende (1976) reported a reduced basic activity (Grundaktivität) in mild injuries only a few hours after the trauma In his experimental model Ommaya & Gennarelli (1976) could not establish any significant correlation between the EEG findings and the severity of the head injury (visual or computerassisted analysis of EEG)

On the basis of ENG and EEG records Greiner (et al 1966) came to the following conclusions If both EEG and ENG recordings are normal the prognosis is favourable An abnormal ENG obtained soon after injury has no prognostic value but if the abnormality persists even though the EEG has returned to normal the prognosis is poor Finally if both EEG and ENG are markedly abnormal the prognosis is very unfavourable Moser (1977) did not find any correlation between the EEG and ENG after trauma

III GOAL OF THE STUDY

The purpose of this study was to clarify

- the occurence of vertigo or dizziness and their correlation with ENG findings
- whether the posttraumatic vestibular and cochlear disturbances are to be determined as peripheral, central, or combined and their reversibility during the six month follow up period
- the correlation of vestibular and cochlear disturbances with the duration of unconsciousness and the patient's age
- the correlation of vestibular and cochlear disturbances with the region of the skull involved in the trauma
- the occurence of spontaneous and positional nystagmus after mild traumas
- the changes in nystagmus thresholds and in the results of postrotatory tests during the six month follow up period
- the correlation of EEG disturbances with the duration of unconsciousness and ENG findings

IV MATERIAL

The material consisted of 82 patients (50 males and 32 females) suffering from acute mild head injury The maximum duration of unconsciousness was two hours Patients suffering from intracranial hemorrhages or temporal bone fractures were excluded The patients also met the following requirements no neurological, ear or vestibular diseases, no ototoxic medication, no occupational hearing loss, nor any previous head or whiplash injuries All patients were free from all alcoholic beverages and all medication at least 3 days prior to the tests

The patients were treated primarily at the

Table 1 DISTRIBUTION OF THE HEAD INJURY PATIENTS AND CONTROL SUBJECTS ACCORDING TO AGE AND SEX

Age	Patients					Controls				
	Males		Females		Tot	Males		Females		Tot.
	No	%	No	%	No	No.	%	No.	%	No
10 – 20	16	32 0	8	25 0	24	7	36.8	3	27.2	10
21 – 30	12	24 0	6	18.8	18	4	21.1	2	18.2	6
31 – 40	5	10 0	6	18 8	11	2	10.5	2	18.2	4
41 – 50	9	18 0	3	9 4	12	3	15.8	1	9.1	4
51 – 60	2	4 0	5	15.6	7	1	5 3	1	9.1	2
61 – 70	6	12 0	2	6 2	8	2	10.5	1	9.1	3
71 –	-	-	2	6 2	2	-	-	1	9.1	1
	50	100 0	32	100 0	82	19	100 0	11	100.0	30
Mean age	36 8 SD 19 6		31 7 SD 17.5			36.3 SD 20.7		30.9 SD 17.1		
	34.2 SD 17.4					32.9 SD 18.3				

University Central Hospital of Turku during tho years 1975—1978. The ages of the patients ranged from 13 to 75 years (mean age 34.2 SD 17.4). The age and sex distributions of the material are shown in table 1.

The control series consisted of 30 healthy persons (19 males and 11 females) whose ages varied from 10 to 72 years (mean age 32.9, SD 18.3). They all had normal hearing with no manifested neurological ear or known vestibular diseases, no ototoxic medication, and no previous head injuries (table 1).

V METHODS

1 Case history

The following facts were carefully gathered mechanism of head injury, duration of unconsciousness, posttraumatic amnesia vertigo or dizziness, headache, nausea and vomitus, nervousness sleeplessness inability to concentrate impaired memory, and intolerance to alcohol

2 Clinical examination

The first examination was made on an average of 24 hours after the trauma (0—4 days), the follow up examinations took place at one and six months after head injury The follow up examination at 1 month after trauma was attended by 81/82 patients, at six months the attendance figure was 75/82 The patients who did not attend the follow up examinations were free of symptoms and were therefore unwilling to attend the rather strenuous examinations (table 2)

Table 2 EXAMINATION SEQUENCE

0 – 4 days after head injury	1 month after head injury	6 months after head injury	Controls
Case history	Symptoms	Symptoms	Anamnesis
Clinical examination	Clinical examination	Clinical examination	Clinical examination
ENG tests — PETT — OKN — PN, SN — caloric tests — OFI — rotatory tests	ENG tests — PN, SN — rotatory tests	ENG tests — PETT — OKN — PN, SN — caloric tests — OFI — rotatory tests	ENG tests — PETT — OKN — PN, SN — [illegible] — OFI — [illegible]
Audiogram (pure tone)		Audiogram (pure tone)	[illegible]
Filtered speech test		Filtered speech test	
EEG		EEG	

Normal careful ENT and neurootological tests were made Spontaneous (SN) and positional (PN) nystagmus to both lateral positions and headhanging were studied in the dark room with Frenzel s glasses (Nylen 1950 Cawthorne 1954 Aschan et al 1956) The function of each cranial nerve was tested separately Romberg Unterberger and walking blind folded were carried out to establish cerebellospinal and vestibulospinal functions Finger to-nose and pronation supination tests were performed as indications of cerebeller function

3 ENG tests

ENG tests were performed in a darkened room by employing an AC direct ink writing amplifier (Elema Mingograph 34) except for the rotational tests in which a built in AC amplifier (Polman Mod 11) was utilized The time constant was 2 seconds upper limit 70 Hz

Complete darkness was attained by wearing Welder s goggles in a darkened room Disposable silverchloride ECG electrodes were employed Two electrodes were located as near the outer canthi of the eyes as possible A reference electrode was placed on the skin of the forehead The skin was cleaned with ethanol and electrode paste was utilized to improve the contact

Mental arithmetics were used and the patients were told to keep their eyes open and to look straight forward (Torok 1970) in order to avoid central gaze nystagmus (Aschan et al 1956 Kornhuber 1969 Torok 1970 Scherzer 1975) The patients were kept in the darkened room about 20 minutes to achieve a stabile corneoretinal potential (Munthe 1965) the calibration (10 degrees) was performed as often as possible to eliminate the error caused by fluctuations of the corneoretinal potential (Aantaa 1970)

— spontaneous (SN) and positional nystagmus (PN)

Spontaneous and positional nystagmus were recorded by ENG in total darkness with eyes closed as well as with eyes open in supine and both lateral positions

— caloric tests

Caloric tests were performed in total darkness by wearing Welder s goggles The subject lay supine on a bed with his head raised some 30 degrees above the horizontal thus bringing the lateral semicircular canal into a vertical position (maximal sensitivity to thermal stimuli) The stimulus sequence was 44°C left 44°C right 30°C left and 30°C right with an irrigation time of 30 seconds (5 ml/s) After 70 seconds the subject was told to fix his gaze on a light spot in the ceiling If the nystagmus amplitude or the eye speed of the slow phase (after fixation/before fixation) was less than 50 per cent the result was considered normal (Demanez & Ledoux 1970)

If the lateral difference was greater than 20 per cent the result was interpreted as canal paresis (CP) and directional preponderance (DP)

— pendular eye tracking test (PETT)

Pendular eye tracking test (PETT) was considered to be normal if at least one normal sinuscurve was found (frequency 1/3 Hz)

— optokinetic nystagmus (OKN)

Optokinetic nystagmus (OKN) was provoked by moving black stripes horizontally in the centre of the visual field Alternative velocities of 20°/s 30°/s 40°/s and 50°s left and right were used in this sequence OKN was considered to be pathological if the side difference exceeded 20 % without SN and if dysrhytmia was demonstrable already at 30°/s in either direction

The result was regarded as indicating a central ENG disturbance when PETT and/or OKN and/or 2 of 4 OFI were ≧ 50 %

— rotatory tests (nystagmus thresholds postrotatory nystagmus)

The tests to establish the nystagmus threshold for angular acceleration and deceleration and postrotatory nystagmus were carried out with Polman's rotating chair (mod 11 e 111) The chair was accelerated by 0,2°/s² over a 120-second period which was followed by a 120-second period of constant velocity Deceleration by 0,2°/s² then followed and the test was carried on with progressively increasing values of angular acceleration and deceleration (0 3 — 0 4 — 0 6 — 0 8 — 1 0°/s²) until a clear nystagmus responce was noticed The lowest accelerations (decelerations) needed to produce the nystagmus were set as thresholds If spontaneous nystagmus existed the nystagmus threshold was set at the point where the direction of SN changed

The subject was sitting so that the vertical body axis corresponded to the rotational axis of the chair The head was tilted 30° forward to bring the lateral semicircular canals to match the plane of rotation During the examination the patient was kept alert (Collins & Guedry 1961 Torok 1970 Barr et al 1976) The recordings were carried out in darkness with *open* eyes by using Welder's goggles (Decher 1965 Tibbling 1970 Tjarnstrom 1973 Mulch & Bonnier 1976) None of the patients or the controls showed thresholds of angular acceleration or deceleration greater than 1°/s²

The postrotatory tests were performed by accelerating the chair at 1°/s² to 60°/s (Claussen 1975) and then abruptly stopping (1/3 s) The maximal eye speed the total amplitude and the duration of postrotatory nystagmus were measured If the lateral difference was greater than 30 per cent the result was regarded as pathological (Jung 1953 1954 1964 Lange & Kornhuber 1962)

4 Audiological evaluation

Trained audiometrisians examined the pure tone audiograms by employing the Madsen OB 60 audiometer The audiometer was calibrated according to ISO R 389 in 1964 Also the filtered speech tests were performed and analyzed to clarify central hearing losses (Palva 1965) Air conduction thresholds were corrected with mean socioacusis in ten year periods according to Palva & Jokinen (1970)

5 EEG examination

The EEG recordings were performed conventionally by using the 10—20 system in attaching the Ag/AgCl electrodes and by carrying out a 30-minute recording with a 16-channel Elema EEG apparatus The recordings were made by a trained EEG nurse The patient was kept awake and was aroused immediately if he was on the point of falling asleep Photostimulation was also included in the examination The recordings were made

a primarily (0—4 days after trauma) and
b during the follow up (six months after trauma)

The same clinical neurophysiologist carried out the EEG analysis for all curves EEG was evaluated for normality in consideration of all the pathological findings established and four different grades were used (0 = normal 3 = markedly deviating) Also the over all disturbance the average alpha frequency as measured several points of the specimen, the accentuation of theta and delta as compared with normal the intensity of the fluctuations occurring in alertness asymmetric findings pathological excitability and H reaction in blinking light were evaluated All these variables were graded according to their intensity (0 = normal 1 = doubtful finding 2 = obvious finding 3 = marked finding) and punched into computer cards

6 Statistical analysis

The statistical analysis of the material was performed at the Computing Centre University

of Turku, using DEC 10 and Univac 1108 computers

The statistical significance of differences between two means was tested by using the Student's t test after the application of the F test

The statistical significance of differences between several means was tested by employing the analysis of variance

The statistical significance of differences between observed grouped distributions was tested by using the x^2-test In fourfold tables Yates' correction for continuity was used

In a comparison of two frequencies the method based on binomial distribution was employed The test quotient showed however, approximately normal distribution

The following statistical significance limits were used

*	p 0 05	almost significant
**	p 0 01	significant
***	p 0 001	highly significant

VI RESULTS

1 Mechanisms of trauma

In 47 6 % (39/82) of the material the head injury was caused by a traffic accident The second commonest cause was an accidental fall, violent assault and occupational accident were on the third and fourth places in the causation (table 3)

Table 3 THE MECHANISM OF HEAD TRAUMA

	No	%
TRAFFIC ACCIDENT	39	47 6
ACCIDENTAL FALL	17	20 7
VIOLENT ASSAULT	11	13 4
OCCUPATIONAL ACCIDENT	10	12 2
SPORTS ACCIDENT	2	2 4
SOME OTHER CAUSE	3	3 7
	82	100 0

2 Duration of unconsciousness and posttraumatic amnesia

The patients were (according to the dura (table 4) Group I c whose period of uncon than 15 minutes, in gro patients, the duration o 16—30 minutes, and gro of all 25 patients whose ousness exceeded 30 minu posttraumatic amnesia incr ing unconsciousness, so tha (21/25) with unconscio minutes, suffered from p (table 4)

Table 4 THE DURATION OF UNCONSCIOUSNESS AND AMNES[illegible]

DURATION OF UNCONSCIOUSNESS (min)	No of patients	%	Posttraumatic amnesia No
0 — 15	24	29.3	5
16 — 30	33	40.2	17
31 — 120	25	30.5	21
	82	100.0	4[illegible]

3 Posttraumatic sequelae

All patients (100 %) complained of a episodic or constant headache primarily after trauma (table 5 fig 1) One month later the symptom was present in 54 3 % only (44/81), and 6 months after trauma the percentage had dropped to 40 0 % (30/75) 16 7 % (5/30) of the control subjects complained of an episodic headache Nausea disappeared soon after the trauma Vertigo or diffuse dizziness were primary complaints after trauma in 78 0 % (64/82) of the patients One month later only 43 2 % (35/81) suffered from vertigo/dizziness, and 6 months after the trauma the percentage had dropped to 20 0 % (15/75) There were no complaints of vertigo/dizziness among the controls Also nervous trouble, inability to concentrate, insomnia impaired memory, and alcoholic intolerance were common posttraumatic complaints primarily and one month after head injury (table 5, fig 1) All patients had regained their working ability and returned to their previous work six months after head injury The duration of posttraumatic working disability was on the average 16 7 days

Table 5 EPISODIC OR CONSTANT SYMPTOMS AFTER HEAD INJURY

SYMPTOMS	0 – 4 days after head injury (82 pat) No	%	1 month after head injury (81 pat.) No	%	6 months after head injury (75 pat) No	%	Control subjects (30) No	%
HEADACHE	82	100 0	44	54 3	30	40 0	5	16 7
NAUSEA, VOMITUS	70	85 4	4	4 9	-	-	-	-
DIZZINESS, Vertigo	64	78 0	35	43 2	15	20 0	-	-
NERVOUSNESS	40	48 7	30	37 0	8	10 7	2	6 7
DIFFICULTY SLEEPING	42	51 2	25	30 9	14	18 7	3	10 0
INABILITY CONCENTRATE	51	62 2	30	37 1	11	14 7	2	6 7
IMPAIRED MEMORY			42	51 9	16	21 3	4	13 3
ALCOHOLIC INTOLERANCE			26	34 7	8	10 6	-	-

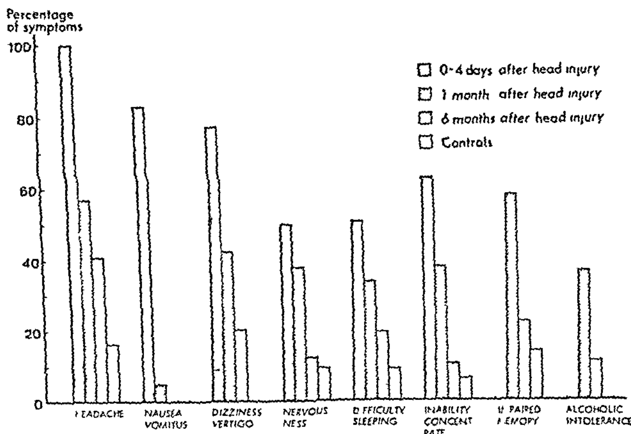


Fig 1 EPISODIC OR CONSTANT POSTTRAUMATIC SYMPTOMS

4 Clinical examination

Romberg's test proved pathological in 61 patients (74.4 %) primarily after trauma after one month there were 35 pathological findings (44.4 %) and after six months the corresponding figure was 17 (22.7 %) (table 6)

Unterberger's test gave pathological results primarily after trauma (0—4 days) in 49 cases (59.8 %) one month later 36 patients (44.4 %) displayed pathology and six months after head injury there were 17 pathological findings (22.7 %) (table 6)

Walking blindfolded yielded pathological results in 43 cases (52.4 %) primarily after head injury and 6 months after trauma there were 10 pathological findings (13.3 %)

Twenty three patients (28.0 %) failed to perfom the *finger-to-nose test* successfully primarily after trauma After one month there were only six failures (7.4 %) and six months after head injury all patients performed this test successfully

Diadochokinesis test gave pathological results primarily after trauma in 21 patients (25.6 %) One month later there were still six (7.4 %) pathological findings but six months after trauma all patients and controls gave normal results (table 6)

Cerebral nerve status Peripheral facialis paresis was observed in one patient who, however, recovered fully within the six-month follow up period Sensibility disturbances due

Table 6 CLINICAL EXAMINATION

	0 — 4 days after head injury		1 month after head injury		6 months after head injury	
	No	%	No	%	No	%
ROMBERG						
no information	9	11.0	-	-	-	-
normal	12	14.6	46	56.8	63	84.0
pathological	61	74.4	35	43.2	12	16.0
	82	100.0	81	100.0	75	100.0
UNTERBERGER						
no information	11	13.4	-	-	-	-
normal	22	26.8	45	55.6	48	77.3
pathological	49	59.8	36	44.4	17	22.7
	82	100.0	81	100.0	75	100.0
WALKING BLIND-FOLDED						
no information	14	17.1	-	-	-	-
normal	25	30.5	57	70.4	65	86.7
pathological	43	52.4	24	29.6	10	13.3
	82	100.0	81	100.0	75	100.0
FINGER TO NOSE TEST						
no information	8	9.8	-	-	-	-
normal	51	62.2	75	92.6	75	100.0
pathological	23	28.0	6	7.4	-	-
	82	100.0	81	100.0	75	100.0
DIADOCHOKINESIS (PRONATION-SUPINATION TEST)						
no information	8	9.8	-	-	-	-
normal	53	64.6	75	92.6	75	100.0
pathological	21	25.6	6	7.4	-	-
	82	100.0	81	100.0	75	100.0

to bruises on the facial skin were recorded in six cases Permanent ptosis was found in two patients even here bruises were obviously responsible for the disorder Gustometry indicated impaired taste functions primarily after trauma (over 100 mA) in four patients

SN or PN primarily after trauma was observed with *Frenzel s glasses* in 25 patients 42 7 %) in the supine or both lateral positions (table 7), in 8 of these cases (9 4 %) the type of nystagmus was established as being BPN After one month nystagmus was recorded in 13 patients (29 5 %), and 6 months after head injury in 5 cases (6 7 %)

No pathological results were obtained in any of the tests above for the control subjects

Table 7 NYSTAGMUS AFTER HEAD INJURY OBSERVED WITH FRENZEL S GLASSES

	0 — 4 days after head injury		1 month after head injury		6 months after head injury		Controls	
	No	%	No	%	No	%	No	%
NO NYSTAGMUS	57	69 5	68	83 9	70	93 3	30	100.0
HORIZONTAL	14	17 1	8	9 9	2	2 7	-	-
VERTICAL	11	13 4	5	6 2	3	4 0	-	-
	82	100.0	81	100 0	75	100 0	30	100 0

5 Electronystagmographic findings

— spontaneous and positional nystagmus

Measurable (2°/s) spontaneous or positional nystagmus could be established in 36 6 % (11/39) of the controls The mean angular velocity of the slow phase of nystagmus was 3 4°/s and in none of the control subjects did it exceed 7°/s (tables 8 and 9)

Primarily after trauma 68 3 % (56/82) of the patients displayed SN and/or PN One month after trauma SN and/or PN could be established in 55 3 % (44/81), and 6 months after head injury in 52 0 % (39/75) of the patients No statistically significant differences between the two groups were demonstrable at one or six months after trauma (table 8) The only statistically significant difference between the patients and the controls in posttraumatic SN and/or PN could be established primarily after head injury (table 8)

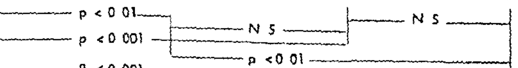
The mean angular velocity of the slow phase was primarily 7.3°/s which is a statistically significantly ($p < 0 01$) higher value than one month later (5 0°/s) After this the decrease in the angular velocity of the slow phase is no longer statistically significant The angular velocity of the slow phase for the controls was 3 4°/s (table 8)

Table 8 SPONTANEOUS AND POSITIONAL NY AGMUS AND MEAN EYE VELOCITY OF THE SLOW COMPONENT (°/s)

	0 – 4 days after head injury				1 month after head injury				6 months after head injury				Controls			
	No	%	Mean (°/s)	SD	No	%	Mean (°/s)	SD	No	%	Mean	SD	No	%	Mean	SD
DIRECTION CHANGING (Nylen I)	10	12 2	6 5	3 2	6	7 4	4 8	2 4	8	10 7	4.1	2 3	3	10 0	2 9	2 2
DIRECTION FIXED (Nylen II)	12	14 7	7 2	4 6	13	16 1	5 5	3 6	12	16 0	4 4	2 8	4	13 3	3 6	3 3
IRREGULAR (Nylen III)	9	10 9	6 6	3 7	5	6 2	4 4	2 5	4	5.3	4 3	2 7	1	3.3	2 7	2 5
SPONTANEOUS NYSTAGMUS	25	30 5	7 6	4.9	20	24 6	5 1	3 2	15	20 0	4 2	2 9	3	10 0	3 8	2 2
	56	68 3	7 3	4 4	44	55 3	5 0	2 9	39	52 0	4 2	2 7	11	36 6	3 4	2 4
NO NYSTAGMUS	26	31 7	-	-	37	44 7	-	-	36	48 0	-	-	19	63 4	-	-
TOTAL	82	100 0			81	100 0			75	100 0			30	100 0		

N S
N S
N S
N S
p < 0 01

7 3	4 4	5 0	2 9	4 2	2.7	3 4	2 4



When 7°/s is employed as the threshold of the angular velocity of the slow phase, SN and */or PN can be established primarily after* trauma in 30 5 % (25/82) of the patients One month later there were SN and/or PN findings in 19 2 % (16/81), and 6 months after trauma 8 0 % (6/75) of the patients showed PN and/or SN exceeding 7°/s (table 9)

Table 9 Spontaneous and/or positional nystagmus (≧ 7°/s) after head injury

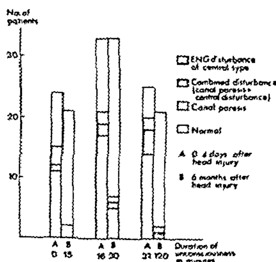
	0 — 4 days after head injury No	%	1 month after head injury No	%	6 months after head injury No	%	Control subjects No	%
SPONTANEOUS AND/OR POSITIONAL NYSTAGMUS (≧ 7°/s)	25	30.5	16	19.8	6	8.0	-	-
NO NYSTAGMUS	57	69.5	65	80.2	69	92.0	30	100.0
TOTAL	82	100.0	81	100.0	75	100.0	30	100.0

— caloric tests

Directional preponderance (DP) could be established in caloric tests primarily (0—4 days) after head injury in only 3 6 % (3/82) of the patients Six months later there were no findings of DP in these patients No DP could be detected in the controls, either

Unilateral caloric hyperactivity (canal paresis) was observed primarily after trauma in 17 1 % (14/82) of the patients which is statistically significant ($p < 0.01$) more than 6 months later when CP was found in 5 3 % (4/75) only No canal paresis could be demonstrated in the controls The duration of unconsciousness (fig 2) or the direction of the blow to the skull showed no statistically significant correlation with the incidence of CP After an unconsciousness of 0—15 minutes, CP was observed in 16 7 % (4/24) of the patients, after a more prolonged uncons-

Fig 2 ENG DISTURBANCES AND DURATION OF UNCONSCIOUSNESS



ciousness (16—30 minutes) the percentage was 12 1 (4/33) If the unconsciousness exceeded 30 minutes, CP was found in 24 0 % (6/25) of the patients No correlation between the

Table 10 MAXIMAL SLOW PHASE VELOCITY (°/s) IN THE CALORIC TEST (EYES OPEN IN TOTAL DARKNESS)

	0 – 4 days after head injury			6 months after head injury			Controls		
	Mean	SD	No	Mean	SD	No	Mean	SD	No
44° sin	29.4	15.4	82	26.4	12.4	75	21.3	14.1	30
44° dx	31.1	17.6	82	26.2	13.5	75	21.8	15.3	30
30° sin	26.1	10.0	82	21.3	7.7	75	18.4	8.2	30
30° dx	25.7	11.8	82	20.6	7.5	75	18 9	8.9	30
	27.3	13.0	328	23.6	10.3	300	20.3	9.7	120

p < 0.001 (0 – 4 days vs 6 months); N.S. (6 months vs Controls)

duration of unconsciousness and the incidence of CP could be demonstrated primarily and after head injury six months (table 14)

The mean maximal angular velocity of the slow phase in caloric tests (N = 4 × 82 = 328) primarily after head trauma (27 3°/s) was highly signifantly ($p < 0\ 001$) higher than six months after head injury (23 6°/s) (table 10) The mean maximal angular velocity of the controls was statistic ally not significantly different from that of the patients six months after trauma (table 10)

The mean maximal frequency obtained in caloric tests primarily after trauma was 2 3 Hz (SD 0 8) and six months later 2 2 Hz (SD 0 7) The corresponding value for the controls was 1 9 Hz (SD 0 7) The maximal fre quencies did not differ statistically from each other

— PETT OKN and ocular fixation index (OFI)

Pathological PETT was observed primarily after trauma in 35 4 % (29/82) of the patients OKN was pathological in 28 0 % (22/82) and pathological OFI could be esta blished in 42 7 % (35/82) of the patients

OFI (ocular fixation index) was found to be primarily after trauma highly significantly ($p < 0\ 001$) higher (34 1 %) than six months later (15 3 %) (table 11) The mean OFI value after an unconsciousness exceeding 30 minutes (46 8 %) was statistically significantly ($p < 0\ 01$) higher than after a shorted period (0—15 minutes) of unconsciousness (24 1 %) (table 11) After six months no statistically significant differences could be established between these groups (16 1 % and 15 4 %)

Primarily after trauma the OFI values of persons older than 40 years of age (50 0 %)

Table 11 THE DURATION OF UNCONSCIOUSNESS AND MEAN OFI.

DURATION OF UNCONSCIOUSNESS (min)	0 — 4 days after head injury			6 months after head injury		
	Mean OFI	SD	No	Mean OFI	SD	[illegible]
0 — 15	24.1	28.2	24	15.4	20.[illegible]	[illegible]
16 — 30	31.7	26.8	33	14.8	14.[illegible]	[illegible]
31 — 120	46.8**	26.5	25	16.1	[illegible]	[illegible]
	34.1***	28.3	82	15.3	[illegible]	[illegible]

Table 12 POSTTRAUMATIC MEAN OCULAR FIXATION INDEX (OFI) [illegible]

Age	0 — 4 days after head injury		6 months after head injury		Co[illegible]
	Mean OFI (%)	No	Mean OFI (%)	No	[illegible]
< 40	25.4 SD 26.2 Min 0 Max 80	53	11.6 SD 16 7 Min 0 Max 80	50	[illegible]
≥ 40	50.0*** SD 25.3 Min 40 Max 120	29	22.8** SD 16.3 Min 20 Max 120	25	[illegible]
	34.1 SD 28.3	82	15.3 SD 17.3	75	[illegible]

p < 0.001 p < 0.001 p <0[illegible]

were statistically highly significantly ($p < 0.001$) higher than those for patients under 40 (25.4 %) The difference between the

groups pers[illegible] ant even six [illegible] 11.6 % [illegible]

Table 13 PATHOLOGICAL PETT AND/OR OKN AND MEAN OCULAR FIXATION INDEX (OFI)

PETT and/or OKN	0 — 4 days after head injury OFI (%)		Total No	6 months after head injury OFI (%)		Total No
PATHOLOGICAL	45.1**	SD 28.8 Min 40 Max 120	37	32.0	SD 31.1 Min 30 Max 120	6
NORMAL	25.1	SD 24.7 Min 0 Max 80	45	13.9	SD 15.1 Min 0 Max 60	69
	34.1***	SD 28.3	82	15.3	SD 17.3	75

Patients who manifested pathological PETT and/or OKN showed primarily after trauma statistically significantly ($p < 0.01$) higher OFI values (45 1 %) than the other patients (25 1 %) No statistically significant difference could be established between the groups six months after trauma due to the small size of the groups (table 13)

Central ENG disturbances were detected in 59 8 % of the patients primarily after trauma (42 + 7/82) which was highly significantly ($p < 0.001$) more than the corresponding figure six months later by which time the proportion of patients with central disturbances had dropped to 12 0 % (7 + 2/75) (table 14 fig 2)

The mean age of the patients with central disturbances (39 5 years) primarily after trauma was very significantly higher than that of other patients (26 3 years) Six months later no statistically significant differences could be sea (table 15)

Combined ENG disturbances (canal paresis + central disturbances) were found to be present in 8 5 % (7/82) of the patients primarily and in 2 7 % (2/75) after six months The duration of unconsciousness could not be shown to have any statistically significant effect on the occurrence or central ENG-disturbances (after unconsciousness shorter than two hours) either primarily (0—4 days) or after six months (table 14 fig 2)

The direction of the impact on the skull could not be found to correlate statistically with central ENG disturbances primarily nor after six months (table 16 fig 3) After temporal blows central ENG disturbances were observed in all (9) patients after six months all central disturbances in this group had however totally reversed Owing to the small number of the patients in this group no statistically significant differences could however be determined between these and other patients (table 16 fig 3)

Table 14 ENG DISTURBANCES AND DURATION OF UNCONSCIOUSNESS

	0-4 days after head injury					6 months after head injury				
	Duration of unconsciousness					Duration of unconsciousness				
	0-15 No	16-30 No	31-120 No	No	%	0-15 No	16-30 No	31-120 No	No	%
ENG DISTURBANCE OF CENTRAL TYPE	11	17	14	42	51 3	2	5	-	7	9 3
COMBINED DISTURBANCE canal paresis and central type	1	2	4	7	8 5	-	1	1	2	2 7
CANAL PAPESIS	3	2	2	7	8 5	-	1	1	2	2 7
NORMAL	9	12	5	26	31 7	19	26	19	64	85 3
	24	33	25	82	100 0	21	33	21	75	100 0

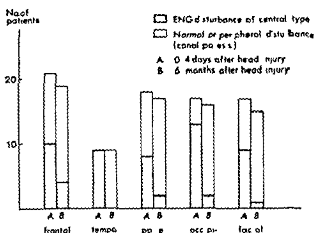
Table 15 POSTTRAUMATIC ENG DISTURBANCES OF CENTRAL TYPE AND AGE

ENG-FINDINGS	0 — 4 days after head injury			6 months after head injury		
	Mean age	SD	No	Mean age	SD	No
ENG DISTURBANCE OF CENTRAL TYPE	39.5***	18.5	49	39.3	17.1	9
				(N.S.)		
ENG NORMAL OR CANAL PARESIS	26.3	12.2	33	33.0	17.8	66
	34.1	17.4	82	34.1	17.1	75

Table 16 LOCATION OF BLOW TO HEAD AND ITS EFFECT ON CENTRAL ENG DISTURBANCES.

Location of blow to head	0 - 4 days after head injury			6 months after head injury		
	ENG disturbance of central type No	%	Total No	ENG disturbance of central type No	%	Total No
FRONTAL	10	47.6	21	4	21.2	19
TEMPORAL	9	100.0	9	-	-	-
PARIETAL	8	44.4	18	2	11.8	17
OCCIPITAL	13	76.5	17	2	12.2	16
FACIAL	9	52.9	17	1	6.7	15
	49	59.8	82	9	12.0	75

Fig 3 ENG DISTURBANCES OF CENTRAL TYPE AND LOCATION OF BLOW TO HEAD



— rotatory tests (nystagmus thresholds, postrotatory tests)

The nystagmus thresholds (right and left) in angular acceleration primarily (0—4 days) (0 28°/s² and 0 27°/s²) or one month after the trauma (0 31°/s² and 0 30°/s²) did not display any statistically significant differences from the corresponding values of the controls (0 28°/s² and 0 29°/s²) (table 17, fig 4) Six months after the head injury the nystagmus thresholds (0 35°/s² and 0 36°/s²) were almost significantly ($p < 0\ 05$) higher than the corresponding thresholds for the control groups (table 17, fig 4) The nystagmus thresholds of angular acceleration and deceleration did not differ statistically significantly from each other, nor could any differences be demonstrated between the two sides (left — right) The age and the sex of the patient or the duration of unconsciousness after trauma had no statistically significant effect on the nystagmus thresholds in angular acceleration or deceleration

The mean maximal postrotatory angular velocity of the slow phase of postrotatory

Table 17 MEAN NYSTAGMUS THRESHOLDS FOR ANGULAR ACCELERATION LEFT AND RIGHT (°/s²)

	0 - 4 days after head injury			1 month after head injury			6 months after head injury			Controls		
	sin	dx	No	sin	dx	No	sin	dx	No	sin	dx	No
MEAN	0.28	0.27	82	0.31	0.30	81	0.35	0.36	75	0.28	0.29	30
SD	0.13	0.10		0.15	0.11		0.17	0.18		0.10	0.11	
MIN	0.2	0.2		0.2	0.2		0.2	0.2		0.2	0.2	
MAX	0.6	0.6		0.8	1.0		1.0	0.8		0.8	0.6	

0–4 days vs 1 month: $p < 0.01$; 0–4 days vs 6 months: $p < 0.001$; 0–4 days vs controls: N.S.; 6 months vs controls: $p < 0.05$

nystagmus right and left (12 9°/s and 13.2°/s) was primarily statistically highly significantly ($p < 0.001$) higher than one month after trauma (9 7°/s and 9 7°/s) (table 18, fig 5) In a comparison with the values obtained 6 months after trauma (8 6°/s and 9 0°/s) the one month values are statistically significantly ($p < 0.01$) higher although the fall during the follow up period is conspicuously retarded towards the end of the period The mean values of the maximal angular velocity of the slow phase in the controls (7 7°/s and 7.2°/s) were not statistically significantly different from the values obtained for the patients six months after trauma (table 18, fig 5)

Fig 4 THE NYSTAGMUS THRESHOLDS FOR ANGULAR ACCELERATION (°/s²) LEFT AND RIGHT

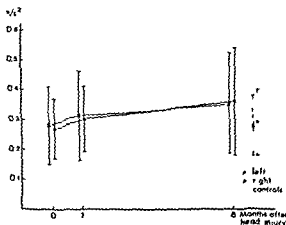


Fig 5 MAXIMAL POSTROTATORY EYE VELOCITY (°/s)

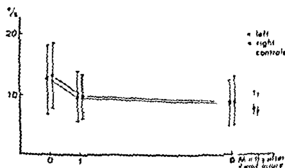


Table 18 MAXIMAL POSTROTATORY EYE VELOCITY (°/s²) LEFT AND RIGHT

	0 - 4 days after head injury			1 month after head injury			6 months after head injury			Controls		
	sin	dx	No	sin	dx	No	sin	dx	No	sin	dx	No
MEAN	12.9	13.2	82	9.7	9 7	81	8.6	9.0	75	7.7	7.2	30
SD	5.9	5.5		4.1	3.7		3.9	4.1		3.0	2.9	
MIN	4.8	3.9		3.7	4.5		2.9	3.6		4.2	3.7	
MAX	42.1	37.8		28.4	30.6		25.9	31.3		35.1	30.6	

0–4 days vs 1 month: p < 0.001; 1 month vs 6 months: p < 0.01; 6 months vs controls: N.S.
0–4 days vs 6 months: p < 0.001; 1 month vs controls: p < 0.05
0–4 days vs controls: p < 0.001

The total amplitudinal values of postrotatory nystagmus primarily after trauma to the left (327°/s) and to the right (321°/s) were also statistically highly significantly ($p < 0001$) higher than the values established one month after trauma (255°/s and 249°/s) (table 19, fig 6) After this the fall of the total amplitudinal values was slower but was statistically still almost significant ($p < 005$) after six months (218°/s and 221°/s) The total amplitudinal values six months after trauma (218°/s and 221°/s) did not differ statistically significantly from the correspond ing values obtained from the control group (177°/s and 155°/s) The duration or the maximal frequency of postrotatory nystagmus of the patients did not differ statistically sig nificantly from those of the controls

Neither the age nor sex of the patient nor the duration of unconsciousness were found to correlate statistically significantly with the maximal angular velocity of the slow phase of postrotatory nystagmus or the total ampli tudinal values

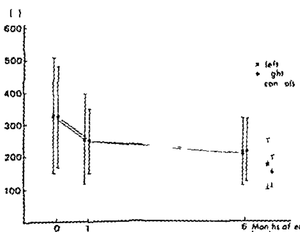


Fig 6 TOTAL POSTROTATORY EYE VELOCITY (s)

6 *Audiological results*

Highly significantly ($p < 0001$) deterio-rated hearing results at 4, 6 and 8 kHz were established for trauma patients primarily and *6 months after trauma when compared with*

Table 19 TOTAL POSTROTATORY AMPLITUDE (o) LEFT AND RIGHT

	0 - 4 days after head injury			1 month after head injury			6 months after head injury			Controls		
	sin	dx	No	sin	dx	No	sin	dx	No	sin	dx	No
MEAN	327	321	82	255	249	81	218	221	75	177	155	30
SD	178	157		143	101		108	103		78	51	
MIN	50	62		45	35		52	60		31	20	
MAX	1200	1150		980	878		670	798		533	620	

p < 0 001 (0–4 days vs 1 month)
p < 0 001 (0–4 days vs 6 months)
p < 0.001 (0–4 days vs controls)
p < 0.05 (1 month vs 6 months)
p < 0.001 (1 month vs controls)
N.S. (6 months vs controls)

the control material (table 20, fig 7) During the 6-month follow up period no significant improvement could be demonstrated in the hearing thresholds (table 20) The hearing impairment was *bilateral* and *sensorineural* No differences between the sexes were observed

The greatest hearing losses were established at 6 kHz immediately after trauma (28 6 dB) At 4 kHz (20 7 dB) and 8 kHz (22 0 dB) mean hearing thresholds did not different from each other The duration of unconsciousness was not found to have statistically significant *effects on the hearing thresholds in the* primary phase (table 21) Age correction was applied to the patients whose unconsciousness exceeded 30 minutes because the mean age of these patients (41 years) was higher than that of other trauma patients The age-corrected hearing thresholds of the patients whose unconsciousness exceeded 30 minutes were 18 5 dB at 6 kHz immediately after trauma If the unconsciousness was shorter than 15 minutes the corresponding hearing threshold was 11.5 dB (table 21, fig 8) In the cases whose unconsciousness was shorter than 15 minutes the hearing threshold underwent the largest corrections (11 3—6 7 dB) Six months after trauma the duration of unconsciousness was found to have no correlation with the age corrected hearing thresholds (table 21)

The direction of the blow to the skull was not observed to have any statistically signific

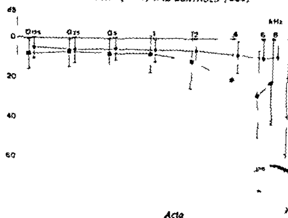


Fig MEAN HEARING THRESHOLDS (dB SD PR MAR LY 0 4 days AFTER HEAD INJURY (•—•) AND CONTROLS (•—•)

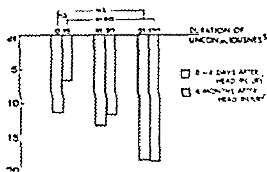
Table 20 HEARING THRESHOLDS (dB) AFTER HEAD INJURY

Hz		Patient group 0 - 4 days after head injury 164 ears	6 months after head injury 150 ears	Reference group 60 ears
125	MEAN	8.4	6.7	5.2
	SD	7.2	6.6	5.3
	MIN	0	0	0
	MAX	25	20	10
250	MEAN	7.8	6.3	6.0
	SD	7.1	6.8	6.4
	MIN	0	0	0
	MAX	20	15	5
500	MEAN	8.0	6.6	5.5
	SD	8.3	8.1	6.0
	MIN	0	0	0
	MAX	25	20	10
1000	MEAN	8.3	7.1	6.0
	SD	9.8	10.5	5.7
	MIN	0	0	0
	MAX	20	15	15
2000	MEAN	11.2	10.6	6.3
	SD	14.2	15.8	6.6
	MIN	0	-5	-5
	MAX	25	30	15
4000	MEAN	20.7	18.8	8.5
	SD	21.7	22.2	8.2
	MIN	0	0	-5
	MAX	60	60	40
6000	MEAN	28.6	26.2	9.7
	SD	22.3	24.2	10.2
	MIN	5	5	0
	MAX	80	85	45
8000	MEAN	22.0	21.2	9.2
	SD	22.3	23.7	10.6
	MIN	0	0	-5
	MAX	75	80	45

Table 21 MEAN HEARING THRESHOLDS AND AGE CORRECTED HEARING THRESHOLDS (at 6 kHz) AND THE DURATION OF UNCONSCIOUSNESS

Duration of unconsciousness	Mean age	SD	0 — 4 days after head injury dB	SD	Age corrected		No	6 months after head injury dB	SD	Age corrected		No
0 — 15	30.0	14 4	21 5	16 2	11 3	9.6	48	16.9	16.2	6.7	8.1	42
16 — 30	32 0	17.8	26.2	21.3	13.3	15.2	66	24.5	23.0	11.7	16.5	66
31 — 120	41 0	18.2	38.5	25.5	18.5	17.0	50	38.3	28.0	18.6	18.9	42
	34.2	17.0	28.6	22.3	14.4	14.6	164	26.2	24.2	12.2	15.9	150

Fig 8 AGE CORRECTED MEAN HEARING THRESHOLDS (at 6 kHz) AFTER HEAD INJURY



ant effect on hearing thresholds at 4, 6, or 8 kHz (table 22, fig 9) The most prominent (20.3 dB) fall of the hearing threshold was recorded with blows to the occipital region, in the facial region the fall of the hearing threshold caused by the impact was the least marked (9 0 dB)

The hearing thresholds of patients with canal paresis were not found to differ statistically significantly from those of other trauma patients at 4, 6, or 8 kHz Nor did the hearing thresholds of patients with manifested central ENG disturbances differ statistically significantly from those of other patients with head injuries

Fig 9 LOCATION OF HEAD BLOW AND AGE CORRECTED HEARING THRESHOLDS (dB) AT 6 kHz

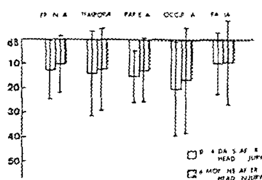


Table 22 LOCATION OF HEAD BLOW AND AGE CORRECTED MEAN HEARING THRESHOLDS AT 6 kHz (dB)

	0 – 4 days after head injury					6 months after head injury				
			Age corrected		No of ears			Age corrected		No of ears
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
FRONTAL	25.7	19.9	12.8	12.5	42	23.3	20.6	10.3	11.7	38
TEMPORAL	32.8	26.8	13.9	17.4	18	27.8	27.3	12.3	16.6	16
PARIETAL	31.3	23.4	15.0	10.8	36	29.6	28.0	12.7	13.0	34
OCCIPITAL	38.1	20.6	20.3	19.4	34	34.4	23.7	16.6	21.6	32
FACIAL	17.5	12.0	9.9	12.8	34	16.7	16.9	9.5	17.2	30
	28.6	21.1	14.4	14.5	164	26.2	23.4	12.2	15.9	150

N.S. (Mean 0–4 days vs. Mean 6 months)

N.S. (Age corrected mean 0–4 days vs. Age corrected mean 6 months)

7 EEG results

Pathological or severely pathological EEG findings were established primarily after head injury (0—4 days) in 53.5 % (30 + 8/71) of the patients which figure was statistically highly significantly ($p < 0.001$) greater than that obtained six months after trauma at wich time pathological findings were recorded in 20.2 % (14/69) of the patients (table 23) No sevely pathological findings could be observed at the follow up examination at six months after trauma The duration of unconsciousness was not found to correlate statistically significantly with the quality of the EEG findings primarily or at six months after trauma (table 23) The patients with manifested central ENG disturbances did not display a greater amount of *pathological EEG findings (16 + 8/43)* than the patients with normal ENG or canal *paresis* (14/28) (table 24) All cases with severely pathological EEG findings (8 patients) also showed central disturbances in the ENG No statistically significant correlations could be established between the EEG and ENG findings at the six month follow up examination, either

The mean alpha frequency primarily after head injury (9.13 Hz) was statistically significantly ($p < 0.01$) lower than that measured six months after trauma (9.67 Hz) The duration of unconsciousness was not found to correlate statistically significantly with alpha frequencies (table 25) Only if the duration of unconsciousness exceeded 30 minutes, was the rise of alpha frequency (1.00 Hz) *statistically* significantly ($p < 0.01$) higher than that after *unconsciousness* of less than 30 minutes

duration (table 25) Only two patients showed falling alpha frequencies Patients with central ENG-disturbances could not be demonstrated to have statistically significantly different alpha frequencies (9 0 Hz) from those of other trauma patients (9 32 Hz) primarily after head injury No statistically significant differences could be established at the six month follow up examination, either, between the two groups (9 44 and 9 68 Hz, respectively) (table 26)

Table 23 EEG AND DURATION OF UNCONSCIOUSNESS

EEG FINDINGS	0 – 4 days after head injury					6 months after head injury				
	0–15	16–30	31–120	Total No	%	0–15	16–30	31–120	Total No	%
NORMAL	5	9	6	20	28.2	11	19	10	40	58.0
MILD PATHOLOGICAL	6	5	2	13	18.3	6	3	6	15	21.8
MODERATE PATHOLOGICAL	6	16	8	30	42.2	2	8	4	14	20.2
SEVERE PATHOLOGICAL	2	1	5	8	11.3	-	-	-	-	-
	19	31	21	71	100 0	19	30	20	69	100.0

Table 25 THE DURATION OF UNCONSCIOUSNESS AND ALPHA-FREQUENCY IN THE EEG

Duration of unconsciousness (min.)	0 – 4 days after head injury	Total No	6 months after head injury	Total No	Mean-frequency difference	α-freq decrease	α-freq equal	α-freq increase	No
0 – 15	9.37 SD 1.26 Min 7 Max 13	19	9.74 SD 1.05 Min 8 Max 13	19	0.31 SD 0.60	2	12	5	19
16 – 30	9.26 SD 1.18 Min 8 Max 12	31	9.54 SD 1.01 Min 9 Max 13	30	0.24 SD 0.83	1	20 (12 + 20 = 32)	9 (5 + 9 = 14)	30
31 – 120	8.71 SD 1.23 Min 7 Max 11	21	9.75 SD 1.02 Min 7 Max 12	20	1.00** SD 0.97	0	8	12**	20
	9.13 SD 1.23 Min 7 Max 13	71	9.67 SD 1.01 Min 7 Max 13	69	0.48 SD 0.88	3	40	26	69

p <0.01 (0 – 4 days after head injury vs. 6 months after head injury)

Table 24 CORRELATION BETWEEN ENG AND EEG

	0 — 4 days after head injury				6 months after head injury			
	A ENG - normal" -canal paresis	B ENG -central type	No	%	A ENG - normal" -canal paresis	B ENG -central type	No	%
NORMAL	8	12	20	28 2	36	4	40	58 0
MILD PATHOLOGICAL	6	7	13	18 3	13	2	15	21 8
MODERATE PATHOLOGICAL	14	16	30	42 2	11	3	14	20 2
SEVERE PATHOLOGICAL	-	8	8	11 3	-	-	-	-
	28	43	71	100 0	60	9	69	100 0

Table 26 CORRELATIONS BETWEEN ENG AND α FREQUENCY IN EEG

ENG-findings	0 — 4 days after head injury			6 months after head injury		
	Mean	α-freq	No	Mean	α-freq	No
ENG — normal — canal paresis	9.32	SD 0 98	28	9 68	SD 1 07	[illegible]
ENG DISTURBANCE OF CENTRAL TYPE	9 00	SD 1 36	43	9 44	[illegible]	[illegible]
	9 13	SD 1 23	71	9 67	[illegible]	

$p < 0 01$

VII DISCUSSION

Many authors regard dizziness or vertigo after head injury as sequelae of disturbances in the peripheral vestibular system this concept is principally based on positional and spontaneous nystagm ndings as well as on caloric tests (Barany ..., Harrison 1956, Preber & Silfverskiold 1957, Cope & Ryan 1959, Lange & Kornhuber 1962, Cawthorne 1964, Barber 1964, McHugh 1966, Araki 1967, Schuknecht 1962, 1969, et al 1973, 1974, Pearson & Barber 1973, McClure et al 1973, et al 1977) Temporal bone fractures have often been included in previous investigations which were mainly retrospective The benign positional nystagmus (BPN) frequently encountered with skull injuries is assumed to be due to otolith dysfunction possibly otolith luxation (Henriksson 1974) No positive histological evidence of this assumption has so far been presented (Schuknecht 1950 Ward 1969, Makishima & Snow 1976) Kotova (1962) and Scherzer (1975) suggest that this might be a combined disturbance whereby an immediate posttraumatic peripheral vestibular disturbance might account for the symptom of dizziness or vertigo Subsequently mainly more diffuse sensations of dizziness might possibly be due to central disturbances

In the present study a investigation of patients with head injuries was conducted The primary posttraumatic (0—4 days) caloric excitability was lowered unilaterally in 17 1 % (14/28) of the patients but after six months the percentage was not higher than 5 3 (4/75) The duration of unconsciousness was not found to correlate with the presece of a peripheral lesion (CP) These results are in accordance with those presented in the investigation of Podoshin & Fradis (1975) who reported significant recovery from canal paresis six months after trauma

This reversible change is apt to corroborate the assumption that lesions in the vestibular nerve rather than in the membranous labyrinth (semicular canals or macula utriculi or sacculi) are responsible for the disturbance The neuron is possibly stretched when the brain moves in its liquid space (Windle et al 1944, Denny—Brown & Russel 1946, Strich 1961, 1969, 1970 Teasdale 1976 Makishima & Snow 1976) Complaints of vertigo were no more frequent among patients with canal paresis than among any other subjects Spontaneous and/or positional nystagmus could not be established more frequently in patients complaining of vertigo than in other cases This provides another indication of the fact that vertigo need not necessarily refer to peripheral lesions

Central ENG disturbances (pathological PETT and/or OKN and/or pathological OFI) were observed immediately (0—4 days) after trauma in 59 8 % (49/82) of the patients 6 months afterwards the proportion was only 12 % (9/75) No corresponding investigations have been published previously in the literature

The duration of unconsciousness did not correlate with the occurrence of central ENG disturbances primarily or 6 months after trauma The mean age of these patients (39 5 years) was highly significantly ($p < 0\,001$) higher than that of patients with no central

disturbances after head injury (26.3 years). Also six months after trauma the mean age of the patients with persistent central ENG disturbances was higher than that of other head injury patients. No central ENG disturbances were observed in the controls. It seems that the time difference of unconsciousness from 15 minutes to 120 minutes is still too short to show quantitative differences in clinical brain injury, a group of patients with severe traumatic unconsciousness could possibly contribute more information.

As previously known, the aged brain tolerates head injury poorly (Carlsson et al. 1968, Heiskanen 1977). Central ENG- disturbances after trauma increased with advancing age also. These patients had also already had earlier subclinical central disturbances possibly in the vertebrobasilar circulation which then were further provoked by the trauma (Schiller & Hedberg 1960, Harrison 1975).

Pearson & Barber (1973) and Ommaya (1976) found that damage to the occipital region proved to be the most perilous to the brain structure. Bandini (1963) traced a greater number of pathological vestibular findings in lateral and multiple injuries than in fronto-occipital lesions. Toglia (et al. 1970) on the other hand could not establish any correlation between the direction of the head injury and the vestibular findings. No statistical differences could be found in the present investigation between lesions in the different regions of the skull and the ENG findings. All 9 patients with injuries in the temporal region were found to have central ENG disturbances which however disappeared totally within the follow up period. This group was, at any rate, too small for statistical processing.

Immediately after trauma the mean OFI values (34.1 %) were very significantly ($p < 0.001$) higher than six months after trauma (15.3 %). This proves that central inhibition after the injury had decreased. The cerebellum, the formatio reticularis and the intermediate areas are the regions of vital importance to central inhibition (Fernandez & Fredrickson 1964, Demanez & Ledoux 1970, Ryu & McCabe 1976, Robinson 1976, Llinas & Wolfe 1977, Hirosaka & Kawakami 1977, Robles & Anderson 1978, Abend 1978, Kotchabhakdi & Walberg 1978). The biochemical and histological changes responsible for ENG disturbances are likely to occur in these structures. The reversible character of the changes is mainly accountable for by the fact that structures were damaged by the shear strain effect and not by total disruption of neurons (Denny—Brown 1946, Strich 1960, Teasdale 1976, Makishima & Snow 1976).

In consideration of the knowledge that PETT and/or pathological OKN indicate a central disturbance (Takahashi 1969, Dix & Hood 1971, Brask 1974, Barr et al. 1976, Stround & Ranchbach 1976, Sakata & Umeda 1976) the OFI values of the patient were also studied. Immediately after trauma they were highly significantly ($p < 0.001$) higher than in other patients (45.1 % and 25.1 %, respectively). This is also apt to support the concept that in central disturbances the OFI values are higher than normal (Demanez et Ledoux 1970). Also the mean maximal amplitudes (27.3°/s) in caloric tests were immediately after trauma (0—4 days) highly significantly ($p < 0.001$) higher than six months later (23.6°/s). No significant changes were demonstrable in the caloric test frequencies during the follow up period.

Horizontal pendular movement of the eyes (Lange & Kornhuber 1962, Scherzer 1975) was established in several patients with head injuries upon rotational and caloric tests. The alertness of the patients had, however decreased but when normal alertness was reattained through conversation the pendular movement tended to disappear.

It remains obscure what really occurs in the brain during trauma. Some investigators suggest that the primary brain injury is cortical (Ommaya et al. 1968, 1976) while others regard brain stem lesions as being primary (Denny—Brown & Russel 1941, Windle et al. 1944, Strich 1961, 1969, 1970, Kirikae et al. 19

Makishima & Snow 1976) Small petechial hemorrhages (Ommaya & Gennarelli 1976, Budzilovich 1976), disturbances in autoregulation (Enevoldsen et Jensen 1977), disorders in the blood brain barrier (Rapport 1976), and the concentrations and metabolism of the neurotransmitters (Hyyppa 1976, 1977) seem to be partly responsible for central disturbances, at least in more severe cases *Ommaya & Gennarelli (1976) were not able* to establish any pathological findings in the histology of severe brain injuries He agrees with Mitchel and Adams (1973) who entertained the opinion that brain stem injuries always involve a diffuse damage to the whole white matter

Ommaya (et al 1968) found in several animal tests that skull rotation is a greater risk to the brain than translation in a straight line Denny—Brown & Russel made a similar observation with animals as early as 1941 Equivalent blows to a freely moving head more frequently cause petechial brain stem hemorrhages than blows to a stable head Consequently, *tension in neck muscles and the soft* tissues of the face evidently have an attenuating effect on rotation Some investigators suggest, on the other hand that translation is the factor responsible for central disturbances (Gurdjian et al 1966 Unterharnschiedt & Higgins 1969, Walker 1973)

Patients with skull injuries often show positional and spontaneous nystagmus which in general is considered to be caused by otolith lesions (Barany 1906 Preber & Silfverskiold 1957, Bergstedt 1961, Bos et al 1963, Barber 1964 Lange et Kornhuber 1962, Harrison et al 1972 1975, Henriksson 1974, Scherzer 1975 Podoshin & Fradis 1975) According to *Bergstedt (1970) SN and PN are due to a lack* of adaptation by the receptors to gravity Nylen (1950) suggested that the posttraumatic disturbance would be localized principally in the vestibular nuclei of the brain stem and maintains that disturbances in central and peripheral system regulation mainly account for PN

Positional nystagmus cannot with propriety be examined by observing the principle 'all or none" as so frequently suggested If 2°/s is set as the limit for the angular velocity of the slow phase, PN or/and SN can be detected in as many as 50 % of normal subjects as well (Mulch & Bonnier 1977) Generally the limit is set as 7°/s (Jongkees et Philipzoon 1964) In the present study it was observed that the *number of SN and/or PN cases in the group* of head trauma patients was not statistically different from that in the control group, one and six months after head injury if the limit was se at 2°/s No significant decrease was observed 6 months after trauma in the occurrence of SN or PN, where as, the slow phase angular velocity of PN and SN was highly significantly ($p < 0\,001$) greater primarily (7 3°/s) than 6 months later (4 2°/s)

No statistical differences between the incidences of Nylen's three types of nystagmus (I—III) and the so-called benign positional nystagmus (BPN) could be established in trauma patients at various points of time *Preber & Silferskiold (1957) and Harrison (et al 1972 1975)* maintain that the localization of the lesion cannot be made on the basis of spontaneous and positional nystagmus only Conventionally, positional nystagmus of type I, or type III is considered to refer to a central disturbance (Nylen 1950 Lange et Kornhuber 1962), and type II is associated with a peripheral disturbance particularly if vertigo of brief duration is involved (Schuknecht 1962, Lange et Kornhuber 1962 Barber 1964, Harrison et al 1972, 1975 McClure et al 1973, et al 1977) According to the present study the division presented above cannot however, be used as a criterion when determining the location *of the lesion*

On the basis of the observations made about positional and spontaneous nystagmus it seems evident that in posttraumatic cases the quantity rather than the quality of the nystagmus is decisive and the disturbance is to be considered central (reduced inhibition) Thus the differences between the peripheral sides emer

ge more easily Also a pathological OPT and/ or PETT and/or OKN refer to central dis turbances particularly in the brain stem

Rotational tests confirm that there is a post traumatic central disturbance apparently to be defined as reduced central inhibition in the first place Immediately after trauma (0—4 days) the maximum amplitude (327°) of post rotatory nystagmus was highly significantly ($p < 0.001$) higher than at one month after trauma (225°) After this the total fall of the amplitude was no longer significant No sig nificant difference could be demonstrated in the total amplitude values at six months or a comparison of head injury patients and the controls The mean values of the maximum amplitude of postrotatory nystagmus were cor respondingly higher primarily after trauma and became almost entirely normal within the six month follow up period No significant difference could be established in the maxi mum frequencies of postrotatory nystagmus nor in the duration of nystagmus between the patients and the reference group Ranacher (1977) maintains that the duration of nystag mus has no quantitative or qualitative role as a reflector of the vestibular system

Montandon (et al 1972) observed a post traumatic rise in nystagmus thresholds and this is now generally regarded as an indication of peripheral disturbances (Decher 1965 Wilmot 1966 Virolainen 1972) Normal thresholds in various populations range bet ween 0 2 and 0 8°/s² depending on the sen sitiveness of the apparatus (Montandon 1960 Virolainen 1972 Laitakari 1977) Immediately after trauma the mean nystagmus thresholds in linear accelerations was 0 28°/s One month after trauma the mean value was 0 31°/s² and thus showed a significant rise ($p < 0.01$) The nystagmus threshold in linear acceleration six months after trauma (0 35°/s²) was highly sig nificantly ($p < 0.001$) elevated in comparison with the values at one month after trauma (0 31°/s) The mean nystagmus threshold of the control group (0 28°/s²) showed a nearly significant difference in comparison with the values of the trauma patients at six months after injury (0 35°/s²) The nystagmus thresh old both in linear acceleration and deceleration in both directions at various time points were statistically equivalent thus indicating the precise calibration of the apparatus The findings are in accordance with those made by Montadon (et al 1972) but tend to imply increased central inhibition rather than de creased peripheral excitability

McClure (et al 1973) prefers rotational tests to caloric tests due to the greater facility in controlling the stimulus and the superior possibilities to act on the end-organ in a more physiological manner Rotational tests are also less exposed to technical errors (Asai et al 1972) McCabe (1973) maintains that better output of central orders can be obtained by stimulating the two end-organs simultaneously (Norre 1977)

Mitigation of posttraumatic responses cannot be actually regarded as a result of habituation In habituation a subject s normal responses are mitigated in repeated control tests (Young & Oman 1969 Greiner et al 1970 Johnson & Torok 1970 Hood 1970 Monnier et al 1970 Pfaltz & Piffko 1972) Kornhuber (1969) maintains that such hyperreactions indicate decreased central inhibition rather than peri pheral hyperexcitability In Scherzer s opinion (1975) this is to be explained by disturbances in the brain stem

Changes in hearing were in accordance with previous results in which posttraumatic im paired hearing has been established at 4 6 and 8 kHz (Schuknecht 1950 1953 1969 Proctor et al 1956 Fradis 1969 Toglia et al 1970 Tos 1971 Lehnhard 1974 Podoshin et al 1975) There is histological evidence of the fact that the extreme sensory and basal cells which are responsible for the 4—8 kHz hearing are more susceptible to damage than the other cells (Schuknecht 1950 Proctor et al 1956 Ward 1969) The mechanism through which damage to sensory cells affects the patient s hearing is as yet unknown (Schuknecht 1950 1953 1960 Makishima & Snow 1975 Lehnhard

1974, Boscher 1976, v Ilberg 1977) In the present study the most severe hearing loss was established at 6 kHz (28 6 dB) At 4 and 8 kHz the impairment of hearing was almost equally severe (20 7 and 22 0 dB, respectively) No significant improvment in hearing could be observed in the follow up examinations at 6 months after trauma In a comparison with the reference group the trauma patients showed highly significantly ($p < 0\ 001$) more impaired hearing at 4, 6 and 8 kHz

Impaired hearing was bilateral and sensori neural as stated previously (Toglia et al 1970) With increasing duration of unconsciousness hearing impairment was found to increase sig nificantly at 4, 6 and 8 kHz With age adjust ments (Palva & Jokinen 1970) the duration of unconsciousness ceases to affect significantly the hearing findings Bilateral hearing changes may be accounted for by the pressure wave theory suggested by Schuknecht (1950 1953) No statistical evidence of different effects of the various skull regions on hearing at 4 6 and 8 kHz could be established Blows to the occipital region however, had a significant lowering effect on hearing thresholds at 6 kHz when compared with blows to the facial region The soft tissues of the facial region apparently tend to mitigate the impact energy (pressure wave)

When studying disturbances of the peri pheral vestibular system (canal paresis) and hearing changes it can be demonstrated that the hearing results of canal paresis patients were not significantly different from those of other patients with head injuries Consequently it may be concluded that posttraumatic peri pheral disturbances in the cochlear and the vestibular systems are independent of each other also the mechanisms are possibly diverse provided the disturbance is not due to a fractured temporal bone

Part of the patients with head injuries under went the filtered speech test to clarify central hearing impairment (Palva 1965 Jerger 1973 Korsan—Bengtsen 1973 Palva & Joki nen 1975 1975 Snow et al 1977) Most of the established changes were transient (Tuohimaa et al 1978) These observations are cor roborated by the investigations of Lehnhard (1974), Makishima & Snow (1976 and Snow et al 1977 who manifested central hearing impairment even in mild skull injuries

Franzen (et al 1958), Bickford & Klass (1966) and Koufen & Dickgans 1978 found with EEG decreased alpha frequencies in EEG recordings taken immediately after head traumas even in mild cases Meyer—Mickelett (1953) could not however, demonstrate any correlation between alpha frequency and the duration of unconsciousness In the present study alpha frequency (9 13 Hz) was observed to rise highly significantly ($p < 0\ 001$) within the six month follow up period up to 9 67 Hz The duration of unconsciousness on the other hand was not found to correlate distinctly with the alpha frequency After unconscious ness exceeding 30 minutes the rise of alpha frequency (1 0 Hz) was significantly ($p<0\ 01$) higher than that of other patients (0 28 Hz) Only 3 patients showed falling posttraumatic alpha frequency

Beaussart (et al 1970) Lorenzoni (1970) and Dichgans et al 1978 established a cor relation between EEG findings and the severity of the head injury Ommaya & Gennarelli (1976) and Moser (1977) on the other hand were not able to demonstrate in their animal studies any correlation between EEG findings and the severity of the head injury Greiner (et al 1966) states that the prognosis is good if EEG and ENG are found to be normal Ab normal ENG findings immediately after trauma have no prognostic value The prognosis is poor if both EEG and ENG are markedly pathological In the present study the pro portion of pathological EEG findings (53 5 %) and that of central disturbances in ENG (59 8 %) were found to be roughly identical No statistical correlation between them could however be demonstrated Only the cases with the most seriously abnormal EEG findings immediately after trauma were found to suffer from ENG disturbances classifiable as central

A normal ENG does not consequently necessarily exclude pathological posttraumatic EEG and vice versa patients with a manifest central ENG *disorder may produce a completely* normal EEG Apparently this is due to the fact that the EEG reflects the state of the cortical and subcortical regions while ENG yields better information about the disorders in the brain stem

These investigations seem to suggest that careful clinical examinations, rotational tests and EEG in addition to balance tests are necessary to clarify posttraumatic vertigo/dizziness and the localization of the lesion

High incidence of central ENG findings immediately after head trauma and the reversibility *of the symptoms supports the theory of* primary brain stem injury in brain concussion unconsciousness, however, the immediate changes in subcortical cortical areas as seen in positive EEG findings show the complex picture of human brain injury Both the clinical treatment of the patients and their economical rights make a very careful and repeated neuro-otological study imperative

VIII CONCLUSION

According to the results of this investigation following conclusions can be drawn

— Central ENG disturbances, principally in the brain stem, are observed in the pathogenesis of the vertigo/dizziness appearing primarily after mild head injuries These disturbances are quickly reversible (less than one month) Peripheral ENG disturbances which are generally associated with skull fractures may induce symptoms for several months after trauma In mild injuries even the peripheral disturbances are reversible

— No difference in the occurrence of vertigo can be demonstrated between central and peripheral vestibular disturbances immediately after trauma

— The duration of unconsciousness or the region affected by the blow cannot be evidenced to have any effects on central ENG disturbances after unconsciousness lasting less than two hours The age of the patient was the most vital factor in the pathogenesis of central ENG disturbances

— Both central and peripheral ENG disturbances were found to be reversible Primarily after head injury the nystagmus responses (caloric tests, spontaneous and positional nystagmus, rotational tests) were very intense indicating a decreased central inhibition due to trauma The high ocular fixation indexes (OFI) established primarily after trauma tend to corroborate opinion contending a decreased cent inhibition

— The findings of spontaneous and positio nystagmus showed that the disturba after mild head injury was quantitat rather than qualitative The angular ve city (7 2°/s) of the slow phase in positio and/or spontaneous nystagmus establis primarily after trauma was very signi antly higher than that found six mon later (4 2°/s)

— Posttraumatic hearing loss was bilate and sensorineural The hearing thresho at 4, 6 and 8 kHz were very significan worse than in the controls No signific rise could be observed in the heari thresholds during the six month follow period after trauma The duration of u consciousness or the region affected by t injury did not correlate with the heari thresholds at 4, 6, or 8 kHz

— The EEG findings did not correlate wi the duration of unconsciousness No co relation could be demonstrated betwe the ENG and EEG findings, eithe Primarily after trauma, the alpha freque cy of the EEG was found to be very si nificantly lower than six months late Central disturbances could be establishe in the ENG only if the EEG was sharpl abnormal

IX SUMMARY

The purpose of this prospective study was to clarify certain neurootological disorders after acute mild head injury The material consisted of 82 head injury patients and 30 control subjects with matched age distribution

The first examination was made at ca 24 hours after trauma (0—4 days) the following two at 1 and 6 months The follow up examinations at one month and six months were attended by 81/82 and 75/82 patients respectively

Careful ENT and neurootological examinations were performed The electronystagmographical (ENG) tests consisted of the pendular eye tracking test (PETT) the optokinetic nystagmus (OKN) spontaneous and positional nystagmus tests caloric tests (ocular fixation index = OFI) and rotational tests (nystagmus thresholds postrotatory nystagmus) Pure tone audiometry and the filtered speech test as well as the electroencephalographic (EEG) recordings were included in the investigation programme

The duration of unconsciousness ranged between 0 and 2 hours Most subjects were traffic accident casualties All patients were capable of returning to working life within the six month follow up period

The neurological tests carried out immediately after trauma gave pathological results in 20—60 % (Romberg Unterberger blind folded walking finger to-nose test and diadochokinesis) The disturbances showed obvious improvement within the follow up period suggesting that the findings were reversible

The principal complaints primarily after trauma were headache (100 %) nausea (85 %) and vertigo/dizziness (78 %) Typical vertigo could be established equally frequently in patients with central disturbances and in those with peripheral disturbances Most symptoms disappeared during the follow up period

Peripheral vestibular disturbances (canal paresis) was established primarily after trauma in 17 1 % but six months later the percentage had decreased to 5 3 The duration of unconsciousness or the region affected by the blow did not correlate with the occurrence of peripheral disturbances

Central ENG-disturbances (pathological PETT and/or OKN and/or OFI) were observed primarily after trauma in 59 8 % but six months later in only 12 % The high age of the patient seemed to be the most significant factor in the pathogenesis of central ENG disturbances The duration of unconsciousness or the region affected by the blow did not correlate with the appearance of central ENG disturbances Neither was any correlation demonstrable between ENG and EEG findings

Spontaneous and/or positional nystagmus was found primarily after trauma in [illegible] % (the angular velocity of the slow phase [illegible] 2 °/s) The positional and spontaneous nystagmus results of the patients were different from those of the controls quantitatively rather than qualitatively The mean angular velocity of the slow phase immediately after trauma was [illegible] s which was very significantly higher [illegible] 0.001) than six months later (4 2° [illegible]

Rotational tests proved (in accordan[illegible]

caloric tests and positional and spontaneous nystagmus) that the nystagmus responses primarily after trauma were more intense than normal This indicates a reduced central inhibition rather than a peripheral hyperexcitability This assumption is also corroborated also by the high OFI values The post traumatic nystagmus thresholds were statistically highly significantly elevated for six months after trauma The values of total and maximal amplitudes in postrotatory nystagmus were primarily after trauma almost twice as high as those of the controls Neither the duration of or the frequency of postrotatory nystagmus were statistically different from those established in the controls

Very significantly ($p < 0.001$) lower hearing thresholds at 4, 6 and 8 kHz were observed in head injury patients than in controls The hearing losses were sensorineural and bilateral No significant improvement could be observed in hearing within the follow up period at 4 6 or 8 kHz The filtration test showed that central hearing losses were almost normalized within the six month follow up period No correlation could be demonstrated between the hearing threshold and the duration of unconsciousness or the region affected by the blow

The pathological EEG findings did not correlate with the duration of unconsciousness Nor was any intercorrelation demonstrable between the ENG and the EEG findings which indicates that EEG is a reflector of cortical ENG of brain stem disturbances The alpha frequency of EEG was found to be significantly ($p < 0.01$) lower primarily after trauma than six months later Central disturbances were established in the ENG only if the EEG was found to deviate sharply from normal

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XII COMBUTER CARDS COMMOTION AND THE VESTIBULAR SYSTEM

CARD 1
1— 3 patient number
4 time of the examination
1 primary (0—4 days)
2 one month after trauma
3 six months after trauma
5 card number
6— 7 age
8 sex
1 male
2 female
9 previous vertigo or dizziness
0 no information
1 none
2 swing ng sensation
3 vertigo
4 dizziness
5 tilting sensation
6 unsteadiness
10 previous headaches
0 no information
1 none
2 once a month
3 once a week
4 every day
11 migraine
0 no information
1 none
2 yes
12 previous ear disorders
0 no information
1 none
2 low pitched ringins
3 high pitched ringing
4 humming
5 buzzing
6 whistling
13 previous ear inflammations left ear
0 no information
1 none
2 several previous inflammations
no present symptoms
14 previous ear inflammations right ear
0 no information
1 none
2 several previous inflammations,
on present symptoms
15 chronic otitis media
0 no information
1 none
2 chronic otitis media in left ear
3 chronic otitis media in right ear
16 mental illnesses
0 no information
1 none
2 yes
17 hypertonia
0 no information
1 none
2 elevated blood pressure
no medication
3 continuing medication for hypertension
18 diabetes
0 no information
1 none
2 treatment with diet
3 treatment with oriblets
4 treatment with insulin
19 heart disease
0 no information
1 none
2 yes
20 arteriosclerosis
0 no information
1 none
2 cerebral sclerosis
3 arteriosclerosis universalis
21 use of alcohol
0 no information
1 none
2 once a month
3 once a fortnight
4 once a week
5 every day
22 use of analgesis
0 no information
1 once a month
2 once a week

3 every day
4 none

23 smoking habits
0 no information
1 no
2 a packet a week
3 a packet a day
4 more than a packet a day

24 nausea due to travelling
0 no information
1 none
2 yes

25 use of ototoxic antibiotics
0 no information
1 none
2 yes (gentamycin, kanamycin, SM)

26 epilepsy medication
0 no information
1 none
2 yes

27 use of other medication affecting the CNS
0 no information
1 none
2 yes (benzodiazepins neuroleptics, tranquilizers)

28 previous head injury (concussion, contusion)
0 no information
1 none
2 more than 10 years ago
3 5—10 years ago
4 1—5 years ago
5 0 5—1 years ago

29 present trauma
0 no information
1 accidental fall
2 assault
3 traffic accident
4 occupational accident
5 sports accident
6 other cause

30 time elapsed between trauma and examination
0 less than one day
1 1—2 days
2 2—3 days
3 3—4 days
4 4—8 days
5 1 month
6 6 months
7 1 year
8 2 years
9 3 years

31 direction of blow
0 no information
1 frontal blow
2 temporal blow, left
3 temporal blow, right
4 parietal blow, left
5 parietal blow, right
6 occipital blow
7 facial blow

32 patient with traumas of different types
1 no
2 yes

33 thorax injury
1 none
2 yes

34 fractures in extremities
0 none
1 left upper extremity
2 right upper extremity
3 both upper extremities
4 left lower extremity
5 right lower extremity
6 both lower extremities
7 all extremities

35 fractures in the cervical vertebrae
1 none
2 yes

36 fractures in the thoricial vertebre
1 none
2 yes

37 fractures in the LS vertebrae
1 none
2 yes

38 duration of unconsciousness
0 no information
1 no unconsciousness
2 0—15 minutes
3 16—30 minutes
4 31—120 minutes
5 longer than 120 minutes

39 retrograde amnesia
0 no information
1 none
2 temorary
3 1—6 hours
4 6—12 hours
5 over 12 hours

40 duration of headache
0 no information
1 no headache
2 under 1 hour
3 1—3 hours
4 3—12 hours
5 12 h—12 days
6 2—8 days
7 under one month
8 1—6 months
9 constant

41 duration of nausea
- 0 no information
- 1 no nausea
- 2 under 1 hour
- 3 1—3 hours
- 4 3—12 hours
- 5 12 h—2 days
- 6 2—8 days
- 7 over 8 days

42 localization of skull fracture
- 0 no information
- 1 no fractures
- 2 mandibular maxillar fracture
- 3 frontal basal fracture
- 4 left temporal fracture
- 5 right temporal fracture
- 6 left parietal fracture
- 7 right parietal fracture
- 8 occipital fracture
- 9 several fractures of varying localizations

43 posttraumatic vertigo/dizziness
- 0 no information
- 1 no symptom
- 2 swinging sensation
- 3 vertigo
- 4 dizziness
- 5 tilting sensation
- 6 unsteadiness

44 positional vertigo
- 0 no information
- 1 none
- 2 episodic in supine position
- 3 constant in supine position
- 4 episodic in left side position
- 5 constant in left side position
- 6 episodic in right side-position
- 7 constant in right side position
- 8 episodic in both side-positions
- 9 constant in both side positions

45 smelling
- 0 no information
- 1 normal
- 2 left anosmia
- 3 right anosmia
- 4 left hyperosmia
- 5 right hyperosmia
- 6 bilateral anosmia
- 7 bilateral hyperosmia

46 visual field in the left eye
- 0 no information
- 1 normal
- 2 reduced medially (nasally)
- 3 reduced laterally (temporally)
- 4 binocular vision

47 visual field in the right eye
- 0 no information
- 1 normal
- 2 reduced medially (nasally)
- 3 reduced laterally (temporally)
- 4 binocular vision

48 double vision
- 0 no information
- 1 none
- 2 to the left
- 3 to the right
- 4 left and right
- 5 upward
- 6 downward
- 7 in all directions except straight forward

49 ptosis, left
- 1 none
- 2 yes

50 ptosis, right
- 1 none
- 2 yes

51 pupillae
- 1 normal
- 2 left miosis
- 3 right miosis
- 4 left mydriasis
- 5 right mydriasis
- 6 bilateral miosis
- 7 bilateral mydriasis

52 pupilla responses to light
- 1 normal
- 2 no response in the left pupilla
- 3 no response in the right pupilla

53 cornea sensibility
- 0 no information
- 1 normal
- 2 reduced in the left eye
- 3 reduced in the right eye
- 4 reduced binocularly

54 facial sensibility to heat
- 0 no information
- 1 normal
- 2 reduced on the left side
- 3 reduced on the right side
- 4 reduced bilaterally

55 facial sensibility to pain
- 0 no information
- 1 normal
- 2 reduced on the left side
- 3 reduced on the right side
- 4 left hyperestesia
- 5 right hyperestesia

56 frowing
- 0 no information
- 1 normal
- 2 failure on the left side
- 3 failure on the right side
- 4 bilateral failure

57 palpebration
0 no information
1 normal
2 failure on the left
3 failure on the right
4 bilateral failure
58 grimacing
0 no information
1 normal
2 left corner of the mouth drooping
3 right corner of the mouth drooping
4 both corners of the mouth drooping
59 lacrimation
0 no information
1 normal
2 reduced, left
3 reduced, right
4 reduced, both
60 tasting in the anterior third area of the tongue
0 no information
1 normal
2 reduced, left
3 reduced, right
4 reduced, bilateral
61 velar reflexes
0 no information
1 normal
2 paresis, left
3 paresis right
62 rest tonus of the tongue eat extension
0 no information
1 normal
2 deviation to the left
3 deviation ti the right
63 shrugging
0 no information
1 normal
2 reduced on the left
3 reduced on the right
64 Romberg s test
0 no information
1 normal
2 staggering
3 tilting forward
4 tilting backward
5 tilting to the left
6 tilting to the right
65 Unterberger's test
0 no information
1 normal
2 sweeping to the left more than 30°
3 sweeping to the right more than 30°
4 slanting forward
5 slanting backward
66 walking blindfolded
0 no information
1 normal
2 slanting to the left
3 slanting to the right
67 finger to-nose test
0 no information
1 normal
2 pathology on the left
3 pathology on the right
4 bilateral pathology
68 diadochokinesis
0 no information
1 normal
2 pathology on the left
3 pathology on the right
4 bilateral pathology

69—70	pure tone agr air	125
71—72	conduction left ear	250
73—74		500
75—76	„	1000
77—78		2000
79—80		4000
	CARD II	
8— 9	pure tone agr air	6000
10—11	conduction left ear	8000
12—13	pure tone agr air	125
14—15	conduction right ear	250
16—17		500
18—19		1000
20—21	„	2000
22—23		4000
24—25		6000
26—27		8000
28—29	pure tone agr bone	250
30—31	conduction left ear	500
32—33		1000
34—35	„	2000
36—37		4000
38—39	pure tone agr bone	250
40—41	conduction right ear	500
42—43		1000
44—45	„	2000
46—47		4000

48 nystagmus in supine position with Frenzel s glasses
0 no information
1 no nystagmus
2 left under 60 sec
3 left over 60 sec
4 right under 60 sec
5 right over 60 sec
6 vertical up under 60 sec
7 vertical up over 60 sec
8 vertical down under 60 sec
9 vertical down over 60 sec
49 nystagmus in left side

with Frenzel's glasses
0 no information
1 no nystagmus
2 left under 60 sec
3 left over 60 sec
4 right under 60 sec
5 right over 60 sec
6 vertical up under 60 sec
7 vertical up over 60 sec
8 vertical down under 60 sec
9 vertical down over 60 sec
50 nystagmus on the right side
with Frenzel's glasses
0 no information
1 no nystagmus
2 left under 60 sec
3 left over 60 sec
4 right under 60 sec
5 right over 60 sec
6 vertical up under 60 sec
7 vertical up over 60 sec
8 vertical down under 60 sec
9 vertical down over 60 sec
51 nystagmus with Frenzel's glasses
after shaking
0 no information
1 nystagmus
2 left under 60 sec
3 left over 60 sec
4 right under 60 sec
5 right over 60 sec
6 vertical up under 60 sec
7 vertical up over 60 sec
8 vertical down under 60 sec
9 vertical down over 60 sec
52—53 nystagmus threshold inaccelerating
rotation to the left
54—55 nystagmus threshold in decelerating
rotation to the right
56—58 total amplitude of postrotatory
nystagmus to the left
59—61 maximal amplitude to postrotatory
nystagmus/10 sec to the left
62—63 maximal frekuency of postrotatory
nystagmus/10 sec to the left
64—65 duration (sec) of postrotatory
nystagmus to the left
66—67 nystagmus threshol in accelerating
rotation to the right
68—69 nystagmus threshold in decelerating
rotation to the right
70—72 total amplitude of postrotatory
nystagmus to the right
73—75 maximal amplitude of postrotatory
nystagmus/10 sec to the right
76—77 maximal frequency of postrotatory
nystagmus/10 sec to the right
78—79 duration (sec) of postrotatory
nystagmus to the right
80 ENG
pendular test
0 no information
1 normal
2 disturbed to the left
3 disturbed to the right
4 disturbed in both directions
CARD III
8 optokinetic test
0 no information
1 normal
2 disturbed nystagmus to the left
3 disturbed nystagmus to the r
4 nystagmus totally disturbed
9 nystagmus in supine position
0 no information
1 no nystagmus
2 nystagmus under 7°/sec to the left
3 „ over „
4 „ under „ to the right
5 „ over „
10 nystagmus in the left side
0 no information
1 no nystagmus
2 nystagmus under 7°/sec to the left
3 „ over „
4 „ under „ to the right
5 „ over „
6 varying direction of nystagmus
11 nystagmus on the right side
2 nystagmus under 7°/sec to the left
3 „ over „ „
4 „ under „ to the right
5 „ over „ „
6 varying direction of nystagmus
12 nystagmus in supine position with
eyes open
0 no information
1 no nystagmus
2 left under 7°
3 left over 7°
4 right under 7°
5 right over 7°
6
13 nystagmus on right side with eyes open
0 no information
1 no nystagmus
2 left under 7°
3 left over 7°
4 right under 7°
5 right over 7°
14 nystagmus on left side with eyes open
0 no information

1 no nystagmus
2 left under 7°
3 left over 7°
4 right under 7°
5 right over 7°
6
15—16 44° left ear ampl
17—18 44° left ear freq
19—20 OFI (ocular fixation index) %
21—22 44° right ear ampl
23—24 44° right ear freq
25—26 OFI
27—28 30° left ear ampl
29—30 30° left ear freq
31—32 OFI
33—34 30° right ear ampl
35—36 30° right ear freq
36—37 OFI
38 effect of hyperventilation
0 no information
1 no effect
2 increased nystagmus
39 effect of holding breath
0 no information
1 no effect
2 increased nystagmus
40 CETP (caloric eye tracking test)
0 no information
1 normal
2 mildly pathological
3 apparently pathological
41 ENG finding
0 no information
1 normal
2 mainly peripheral lesion
3 mainly central lesion
4 totally disturbed
42 pendulation in positional tests
0 no information
1 no pendulation
2 large amplitudinal
3 small amplitudinal
4 varying amplitude
43 'petit ecriture in positional tests
0 no information
1 none
2 yes
44 square wave
0 no information
1 none
2 in certain positions
3 ample square wave
45 filtered speech test
0 right ear discrimination 0— 10 %
1 , " 11— 20 %
2 " , 21— 30 %
3 " " 31— 40 %
4 " " 41— 50 %
5 " , 51— 60 %
6 " " 61— 70 %
7 " " 71— 80 %
8 " " 81—100 %
46 filtered speech test
0 left ear discrimination 0— 10 %
1 " " 11— 20 %
2 " " 21— 30 %
3 " " 31— 40 %
4 " " 41— 50 %
5 " " 51— 60 %
6 " " 61— 70 %
7 " " 71— 80 %
8 " " 81—100 %
47 filtered speech test binaural discrimination
0 0— 10 %
1 10— 20 %
2 21— 40 %
3 31— 40 %
4 41— 50 %
5 51— 60 %
6 61— 70 %
7 71— 80 %
8 81—100 %
48 filtered speech test classification into groups
0 normal
1 monaural pathology, left
2 monaural pathology, right (diff, from left ear over 15 %)
3 binaural pathology
4 generally impaired discrimination
5 monaural pathology, decreased binaural discrimination (10 %)
6 monaural pathology, good binaural discrimination (10 %)

POSTCOMMOTIONAL EEG

CARD IV

1— 3 patient number
4 time of examination
1 primary (EEG)
2 one month after trauma (no EEG)
3 six months after trauma (EEG)
5 card number
6— 7 age
8 sex
1 male
2 female
11 pathology in EEG
0 normal
1 borderline case
2 pathological
3 severely pathological
12 general disorder

0 normal
1 dubious general disorder
2 apparent general disorder
3 severe general disorder
13—14 mean alpha frequency (precision 1Hz)
15 theta and delta
0 not prominent
1 dubious prominence
2 apparent prominence
3 marked prominence
16 alertness
0 stable
1 fluctuation within physiological limits
2 ample fluctuations
3 marked fluctuations
17 asymmetry/focality
0 normal
1 dubious
2 apparent
3 marked
18 asymmetric side
0 normal
1 right
2 left
3 varying
19 irritation
0 normal
1 dubious
2 diffuse or partial eruption
3 organized eruption
20 H response in flashing light
0 no response
1 dubious response
2 distinct response
3 marked response

Acta

OTO-LARYNGOLOGICA

SUPPLEMENTUM 360

Transactions of the XXth Congress of the Scandinavian Oto-Laryngological Society

OSLO, NORWAY, JUNE 19-21, 1978

PRESIDENT

PROFESSOR EMIL STEEN, BERGEN

VICE PRESIDENT

PROFESSOR FINN Ø WINTHER, OSLO

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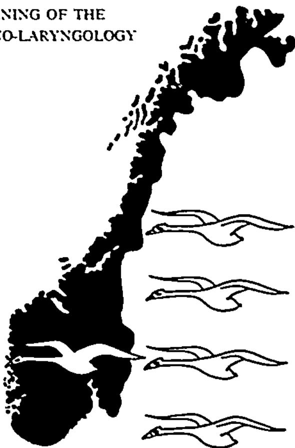
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THE PRESIDENT'S SPEECH AT THE OPENING OF THE XXTH NORDIC CONGRESS OF OTO-RHINO-LARYNGOLOGY

Oslo Concert Hall June 18 1978



This concert house is a symbol of Nordic team work, with a Swedish architecht, A Danish consulting engineer responsible for the acoustics, a Norwegian orchestra and a Finnish con ductor. However, it is not only the house, but the five white Nordic swans on the vignette of the program, and not least, all of us who are gathered here tonight, are also an active and living symbol of Nordic collaboration

Denmark, Finland, Iceland, Sweden and Norway range over a large area of the globe, but we all speak a Nordic language and we can understand one another Let us preserve this advantage at a time when communication and collaboration are more important than ever.

This is the XX Nordic Congree of Oto-rhino-laryngology, quite an impressive number for a congress which is held every third year The reason why these congresses were started was purely professional, but it is equally important to renew old friendships and to meet new friends We try to inform one another of our interests, thoughts and experiences, and of what is in the brewing Not least important, we will meet unbiassed criticism Piet Hein has expressed it nicely in one of his "Gruk"

It is very practical when there is
a cactus leaf among the laurels
then one remembers
they are not to be rested on

The practice of medicine har not always been respected In the time of the Roman Cæsars everyone was free to practice it, but many so called doctors had an unsufficient education or none at all When they did not succeed as doctors, they changed profession and often became gladiators or pall-bearers Martial very maliciously says that the change was not great They were still occupied with killing and burying This is luckily not so today, but we have got other problems

Today the community has introduced rules and regulations for the teaching and the practice of medicine, for the size of our salaries, and for our medical and economic responsibility It is reasonable that the community should have some control because the health sector is becoming more and more expensive Our profession is gradually becoming more socialized But unfortunately some politicians try to socialize by force some things that can never be socialized—and that is research

"The Revolution does not need chemists", cried a political blockhead during the French revolution when Lavoisier's head fell under the axe of the guillotine It is a pity that there are still politicians crying "We do not need

scientists, give us technicians who can solve the problems of today" But today's problems are of no interest tomorrow That is one of the reasons why this congress is so important We have to try to inform one another, exchange thoughts and experiences so that we can solve the problems of tomorrow, together

With the wish that even if we are not wiser after this congress, we will be better informed, I declare the XX Nordic Congress of oto rhino-laryngology opened Quod Bonum Felix Faustum que Sit!

The research which we in the next few days will be informed of, follows a pattern where we try to give life—which is fleeting and changeable, a certain form and firmness so that we can measure and calculate it An artist goes the opposite way, he gives life and spirit to the lifeless materia as our famous Norwegian pianist Kjell Bækkelund now will do

Emil Steen
President

Acta Otolaryngol, Suppl 360 9–12, 1979

PATHOGENESIS OF ALLERGIC RHINITIS

Niels Mygind

From the Otopathological Laboratory Rigshospitalet Copenhagen Denmark

When pollen is sprayed into the nose of a hay fever patient, sneezing starts one minute later, followed by hypersecretion and blockage This is a typical IgE-mediated, type I allergic reaction, causing symptoms which can be treated by specific hyposensitization (immunotherapy)

This article is a short and simplified presentation of the pathogenesis of type I allergic reactions For more detailed information the reader is referred to review articles (Okuda, 1977, Mygind, 1978*a*) and to a recent textbook (Mygind, 1978*b*)

Allergen deposition in the airways

The nose acts as a filter for the inhaled air and the efficacy of this function depends closely upon the size of the particles inhaled Consequently, large particles such as pollen grains are mainly deposited in the nose, causing rhinitis, while smaller particles such as mould spores by pass the nasal filter and cause bronchial asthma As impaction is of importance for deposition of large particles it is, however, possible that some pollen grains may reach the bronchi during slow and deep breathing But it is still an unsolved question, why some pollen allergic patients get asthma in the season Alternative or cooperative possibilities are allergen absorption to the blood course and naso bronchial or laryngo bronchial reflexes

The amount of pollen deposited on the mucous membranes depends obviously upon the pollen content in the air, but the air flow over the actual mucosa is also of significance Thus, physical work will increase nasal deposition and promote rhinitis, while car driving with open windows will promote conjunctival deposition and eye symptoms No significant quantity of inhaled particles will reach the sinus mucosa, while it is highly unlikely that allergy to inhaled particles can cause nasal polyposis

Allergen extraction in airway secretion

Due to the mucociliar clearance, pollen grains will be in contact with the airway mucosa for only 10 to 30 minutes Contrarywise to experimental anaphylaxis in animals, human allergic disease is not characterized by pollen-induced impairment of the mucociliary clearance or by pollen penetration through the epithelial lining However, *in vitro* extraction of pollen grains in nasal secretion has shown that large amounts of allergenic substances can be extracted during the 10 to 30 minutes' stay in the airway secretion, and a maximum concentration is reached after 20 minutes This maximum level is lower when extraction is performed in nasal secretion than in saline, indicating a certain protective function of the airway secretion (Lowenstein & Mygind, 1978)

Allergen penetration of epithelial barriers

It is made probable that a primary defect in the immunological defence, i e a hypo function of secretory IgA, may be of significance for the development of atopic dermatitis and bronchial asthma in infants Admittedly, this is of no importance for the development of hay fever in children and adults, as these patients have a higher level of secretory IgA antibodies to pollen allergens than have normal controls

Neither is there any reliable data to suggest any structural defect in the epithelial barrier as a cause of rhinitis However, all inflammatory reactions will probably increase the epi

scientists, give us technicians who can solve the problems of today' But today's problems are of no interest tomorrow That is one of the reasons why this congress is so important We have to try to inform one another, exchange thoughts and experiences so that we can solve the problems of tomorrow, together

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Neither is there any reliable data to suggest any structural defect in the epithelial barrier as a cause of rhinitis However, all inflammatory reactions will probably increase the epithelial

permeability and with that also the amount of allergen reaching the lamina propria ("submucosa")

Sensitization—the immune response

In some subjects with an inherited predisposition the inhalation of small amounts of allergens will elicit a response in the IgE immune system, which can be discovered by skin-testing This immunological sensitization occurs in 10–20% of the population, but clinical sensitization and disease will only develop in a part of the immunologically sensitized subjects A positive skin reaction, for example to grass pollen, implies a considerably increased risk for subsequent development of clinical hay fever

When the allergenic substances have penetrated through the epithelium they may be ingested by macrophages, which present the processed allergens for the immunocompetent cells, the lymphocytes Only a small number of B-lymphocytes will, upon stimulation with a particular allergen, be transformed into plasma cells, which are capable of forming 000 IgE molecules per second

Stimulation of B lymphocytes and the consequent formation of IgE antibody is under the control of T-lymphocytes, which both partly facilitate the process (helper cells) and partly inhibit it (supressor cells)

Allergen–IgE interaction

The synthesized IgE can be demonstrated in serum as total IgE by RIST and as specific IgE antibody by RAST Distinct from the other immunoglobulin classes, IgE has a special affinity for the surface of mast cells and basophil leukocytes The presence of IgE on the cell membrane can be demonstrated in the blood by histamine liberation test, in the skin by allergen prick-testing and in the airways by allergen provocation of the nose and of the bronchi

The mast cell—a non-specific amplifier system

Allergen–IgE interaction does not itself cause an inflammatory reaction or give rise to symptoms These are due to the immunological activation of amplifier or mediator mechanisms, characteristic for type I allergic reactions, i e mast cell degranulation and release of histamine and other chemical mediators Therefore, the number of mast cells, their content of mediators and their tendency to mediator release are of importance for the allergic symptoms

The mast cell can be stabilized by sodium cromoglycate and possibly also by beta$_2$-adrenergic stimulants Steroids will probably reduce the intracellular content of mediators, but our knowledge is limited on this point

This applies also to mast cell kinetics It is shown that patients with perennial rhinitis have a normal number of mast cells in lamina propria and a significantly elevated number on the epithelial surface This latered cell kinetics may be of significance for the following reasons

1 The increased number of cells on the epithelial surface may increase the non-specific release of mediators

2 There is mounting evidence that the allergen-induced histamine release takes place on or near the surface epithelium

3 Our knowledge about this cell and its fate is important for the development and correct use of mast cell stabilizing drugs, such as sodium cromoglycate

Allergen–IgE interaction on the mast cell surface causes release of histamine, slow-reacting substance (SRS-A), a factor chemotactic for eosinophils (ECF A), and also of other substances While slow-reacting substance is relatively more important in the lower airways, histamine is certainly the most important mediator in rhinitis

Direct and reflectory effect of histamine on effector cells

As blood vessels possess histamine receptors, this mediator has a direct effect on circulation However, it seems unlikely that the glands possess any histamine receptors of significance On the other hand, histamine has an

indirect effect on the glands because it acts on sensory nerve endings in the epithelium (irritant receptors), and induces hypersecretion via efferent nerves in the parasympathetic nervous system As the glands are controlled by and large by the parasympathetic nervous system, stimulation of irritant receptors will be followed by a significant increase in the synthesis as well as in the excretion rate of nasal secretion The blood vessels too possess cholinergic receptors, so the direct histamine effect may be exaggerated by reflex activity The histamine effect on the epithelial irritant receptors will also result in intense itching and sneezing This will via reflex activity cause yet more hypersecretion and vasodilatation

The inflammatory reaction

This is characterized by eosinophilia, vasodilatation, oedema and glandular hypersecretion

Due to diapedesis of eosinophils through the epithelium, eosinophilia is more pronounced in a smear than in a biopsy It is demonstrable in a smear 1–3 hours after allergen challenge and persists for up to 1–3 days

In perennial rhinitis the dilatation of the veins and sinusoids is often accompanied by a constriction of the arterioles, which gives the typical pale bluish appearance of the mucosa In hay fever, nasal blockage is due to vaso dilatation, while oedema may be a contributing factor in perennial disease, especially in nasal polyposis Polyps are devoid of vasoconstrictor nerves which explains the excessive for mation of oedema

It is known that nasal blockage causes a proliferation of goblet cells in the anterior part of the nose but generally speaking exact knowledge of the secretory apparatus in rhini tis is amazingly limited

Late reaction

Following the allergen induced immediate reaction, symptoms may reappear after 4–8 hours in some patients In most cases this late reaction is certainly a direct inflammatory consequence of the allergen–IgE interaction While the direct and the reflectory effects of histamine may dominate in the immediate reaction, cellular infiltration and oedema may characterize the late reaction This type of reaction may be more important for the clinical symptoms than the immediate reaction and will also respond in a different way to treatment

Increased mucosal reactivity

A continuous exposure to allergens will increase the non specific reactivity of the mucous membrane This hyperreactivity is most pronounced in perennial disease, in which it is a highly characteristic feature Therefore, most rhinitis patients will react with symptoms upon exposure to cold air, dust, smoke, washing powder, etc Although the patient and even the physician may call this "allergy" it is a non immunological reaction based on increased irritant receptor sensitivity and increased reactivity of the effector cells Mucosal reactivity can be measured in the clinic by nasal methacholine challenge (Borum, 1978)

Symptoms

The distinction between normality and morbidity is ver vague as regards rhinitis Many subjects have some nasal symptoms, but the perception of these varies considerably Therefore, it is important in the clinic and in particular for research purposes, to quantitate the symptoms thoroughly (hours per day, days per week) and also to get a reliable objective test for the diagnosis of perennial rhinitis, which at present is merely based on the patient's subjective statement For this purpose the simple nasal methacholine challenge may be well suited

Concluding Remarks

Detailed knowledge of the pathogenesis of allergic rhinitis and of the inflammatory reaction is of decisive importance for correct management of these diseases This short review has mainly focused on the basis for the phar-

macological treatment, which in recent years has been considerably improved The immu nological basis for immunotherapy will be dealt with in greater detail in another contribution to this symposium (Aas, 1979)

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DIAGNOSTIC PROCEDURES IN CHRONIC RHINITIS

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Abstract Various methods of diagnosing allergic factors in chronic rhinitis are discussed Among the procedures which aim at detecting specific allergens i e skin testing RAST and nasal provocation tests the last mentioned as they are performed directly on the shock organ have so far been found to give the most accurate picture of clinically dominant allergens and of the intensity and character of the rhinitis However information obtained by analysing the correlations between different procedures is not unanimous As long as test techniques and allergen extracts have not been standardized one particular test cannot be recommended as the method of choice

Causative treatment of allergic diseases can be instituted only after the specific diagnosis has been established In the symptom complex presented by chronic rhinitis it may not be so easy, however, to differentiate etiological factors and evaluate their clinical significance This is especially true of perennial rhinitis where it is seldom possible to reach a conclusive diagnosis by any single test (Aas, 1975)

The various diagnostic procedures which can be employed in allergy work-up are discussed in this paper

1 The value of a good *case history* is so often stressed that we need not elaborate on it here The information which is perhaps best obtained with a well devised questionnaire, can serve as a basis for further diagnostic work

2 There is no characteristic *rhinoscopic finding* in nasal allergy Lividity of the mucosa and nasal polyps may be signs of hypersensitivity but they do not invariably point to a disposition to atopy In an unselected series of 109 patients with nasal polyps, an atopic etiology was confirmed in only 42 patients (Holopainen et al 1978, in press)

3 *The X ray examination* should preferably be made at a time when symptoms are quiescent and findings should be correlated with symptomatology and the rhinoscopic picture A systematic study of 309 patients with perennial and 311 patients with seasonal symptoms showed that mucosal changes in the maxillary sinus were present in 60 and 45% of the two patient groups (Holopainen et al , 1974)

4 *The nasal smear* is also a routine procedure It is particularly useful in differential diagnosis of perennial rhinitis where several etiological factors usually produce the clinical picture The role of tissue, blood and secretion eosinophilia in allergy has attracted much attention (Palva, 1962, Hlavaček, 1963) In the above mentioned series of 109 patients with nasal polyps, parallel examination of eosinophil occurrence revealed a good correlation between tissue and secretion eosinophilia (88%) whereas blood eosinophilia correlated with the other two in only 35% (Holopainen et al , 1978, in press)

5 *Skin testing* is the classic diagnostic procedure aimed at charting the allergic status of the patient In recent years testing by the prick technique has superseded the use of the scratch and intracutaneous methods The prick test is easy to perform and results correlate well with other methods of diagnosing allergy (Stenius Aarniala et al , 1978) When correlating the three skin tests with nasal challenge, we found no significant differences in the sensitivity of the skin tests (Fig 1)

6 Among *in vitro tests* RIST and RAST have gained wider acceptance In nasal allergy, elevated levels of total IgE in the serum were to be found in only 20–30% of patients and correlation with other testing methods was

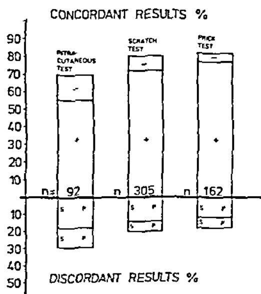


Fig 1 Skin test and nasal challenge correlation in pollen allergy

Table I *Criteria for positivity*

1 Sneezing itching blocking secretion (two of the symptoms present)
2 Change in the colour of the mucosa and swelling
3 Increase in nasal resistance (<25 %)

poor (Holopainen et al , 1976) Specific IgE values, on the other hand, correlate fairly well with skin and nasal test results (Holopainen et al , 1976) As only a serum sample from the patient is needed for the test, it is convenient It is also an advantage that medication and the lergic status of the patient do not affect test results Disadvantages are, on the other hand, the high cost of the test and the fact that test results are available only after several days

7 Direct testing on the shock organ gives the most relevant information about the specificity of mucosal allergy Accordingly, *the nasal provocation test* is considered to give the most exact picture of nasal hypersensitivity (Taylor & Shivalkar, 1971) The test is not very widely used in diagnostic work, because it is associated with several practical difficulties Nasal provocation is time-consuming and may cause unpleasant side effects In addition, difficulties in interpreting results are not uncommon There is as yet no standardized definition of a positive nasal provocation reaction suitable for routine use In our series of some 1 500 controlled provocation tests we have applied the criteria for positivity listed in Table I In these series complications following provocation were rare and it was concluded that the technique of applying the antigen solution locally on the mucosa (Holopainen et al , 1976) and the efforts to use sufficiently diluted allergen solutions in the initial test helped to eliminate complications Unspecific reactions are often reported, especially when glycerinated extracts are used (Haahtela, 1978, 62%) Unspecific reactions were not very frequent in our series, perhaps also on account of the technique used

In a recent comparative study on bronchial, conjunctival and nasal provocation results in the same patients, the nasal test was found to be the most sensitive The nasal mucosa reacted more frequently and a positive reaction was elicited with lower concentrations than those required to provoke conjunctival or bronchial reaction (Stenius-Aarniala et al , 1978, Malmberg et al , 1978)

A comparison of the various diagnostic procedures described above shows that patients who show concordant results in two or three tests do not do so when a fourth method is employed Accordingly, it is not possible to say which diagnostic procedure is more relevant than the other Once the techniques have been standardized, a comparison of testing results with results of treatment could perhaps shed more light on the problem

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THE MOST IMPORTANT ALLERGENS IN ALLERGIC RHINITIS

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Abstract The distribution of allergens found on thorough allergy work up in a series of 770 patients with seasonal and perennial nasal symptoms is reported The percentages of positive reactions to grass tree and herb pollens were equally high (30–40%) but clinically pollens from grasses (Timothy Alopecurus Kentucky Blue and Meadow fescue) birch and mugwort (Artemisia) were the most important In the Nordic countries house dust is evidently the commonest causative agent in perennial rhinitis but the heterogeneous composition of house dust makes it difficult to determine potent allergenic factors Sensitivity to house dust was present in 44% of the patients a positive reaction to mite extract in only 10% Animal danders were not found to be of great importance in allergic rhinitis (13–18%) Reactions to moulds were observed in 9% of the patients

Allergens which invade the upper respiratory mucosa are the most common of the etiologic factors underlying allergic rhinitis On the basis of the time during which allergens are present, we distinguish between seasonal rhinitis ('hay fever'), and perennial rhinitis (symptoms all the year round) Seasonal rhinitis is almost always caused by pollens To what extent moulds cause symptoms during the warmer periods of the year has not been conclusively established, but it may be assumed that they cause mucosal reactions in some atopics from early spring to late summer

Plants which in the Nordic countries are chiefly responsible for nasal symptoms in the period from March to September can be divided into three main groups deciduous trees, grasses, and flowers The allergen distribution among these three pollen groups was studied in a series of 335 patients with seasonal nasal symptoms attending the Ear Nose and Throat Hospital, Helsinki University in the years 1968–76 It was evident that allergens were evenly distributed among trees grasses and flowers, each group including about 40% of the patients

Symptoms appearing in spring and early summer are usually caused by trees Birch, alder and willow were the trees chiefly responsible for symptoms in our material, birch pollen being by far the most important on account of the massive pollination of this tree (Fig 1) Sensitivity to pollens of other broad leaved trees, such as maple, lime and hazel, which are common in the other Nordic countries, but grow only in the south west of Finland, was rare in our material

Allergy to tree pollens is usually associated with hypersensitivity to grass pollens Among the grasses tested, Timothy, Alopecurus, Kentucky Blue grass and Meadow fescue elicited positive reactions the most frequently The percentages appear from Fig 2 and it is evident that the majority of grass sensitive patients reacted to all four species It was, indeed, rare that a patient who gave a positive reaction to, for instance, Timothy, did not react to any of the other three grasses In some

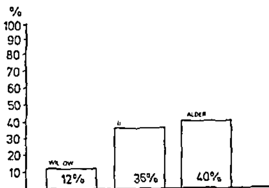


Fig 1 Tree pollens

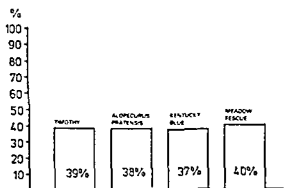


Fig. 2 Grass pollens

patients, positive reactions were recorded for other fairly common grasses, such as Agrostis, Agropyrum, reed etc , but from a clinical point of view these were of minor importance

In late summer, mugwort is the most common cause of seasonal rhinitis in Finland This plant belongs to the composite family and like the broad leaved trees and the grasses it has air pollination Dandelion and Chrysanthemum (oxe-eye daisy) are members of the same family but as they have insect pollination their clinical significance is not nearly as great In the present series of pollen allergy patients, Dandelion and Chrysanthemum also elicited positive reactions in mugwort sensitive patients (Fig 3)

A series of 456 patients with perennial rhinitis symptoms was collected during the same period as the pollen allergy material As there was some overlapping between the two

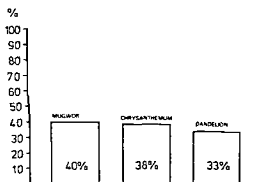


Fig. 3 Pollens of flowers

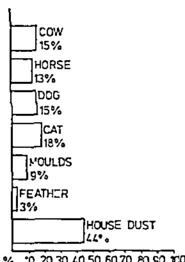


Fig. 4 Distribution of allergens in 456 patients with perennial symptoms

groups the total number of patients was 770 Among the allergens eliciting perennial nasal symptoms house dust was the commonest causative agent On account of the heterogeneous composition of house dust, it is difficult to determine the specific, potent allergen in house dust Mites have been considered to be responsible for the majority of reactions to house dust (Voorhost, 1969, Stenius, 1972) Mite extracts were not used regularly in the earliest part of this study but even when this is taken into consideration, the percentage of positive reactions to mite extract was low (10%) Patients with house dust hypersensitivity may also be allergic to animal danders In the present series of patients, however, animal danders were not very significant allergens (Fig 4)

Positive reactions to moulds were seen in 9% of the patients One explanation of this low percentage may be that indoor humidity is lower in the Nordic countries than in Central Europe and Britain where moulds have been found to play a significant role in house dust allergy

There are obviously variations in the occurrence of allergens in different countries This applies not only to house dust but also to pollens Such circumstances as climate and dura-

tion of flowering seasons must necessarily affect the incidence and prevalence of nasal allergies

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IMMUNOTHERAPY
INDICATIONS AND CONTRA INDICATIONS

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Abstract Immunotherapy in allergic rhinitis should be initiated only when other efforts at elimination and treatment of symptoms prove ineffective The indications become more convincing if symptoms of bronchial hyperreactivity and asthma are also present General preconditions which must be fulfilled prior to treatment are the establishing of a precise diagnosis and that the allergen planned for treatment must play a decisive part in the disease The main contra indications are other concurrent immunological diseases and pregnancy

The indications for treating allergic rhinitis with immunotherapy vary from time to time and from place to place There is no general agreement concerning the course of action such as there is in the treatment of carcinoma of the larynx, for instance

It is difficult to find reliable information about indications in the literature A Medline search covering the last 2 years provided only a few references, mainly dealing with results of treatment, but very little concerning indications The most complete information on the subject is to be found in a book based on a Dome Meda symposium on hyposensitization, edited by Kjell Aas (1977) Further points of view are offered in *Nasal Allergy* by Niels Mygind (1978) This literature and continuing discussions over the years with practising allergologists provide the basis for this article

INDICATIONS

Immunotherapy should never be thought of as the sole way of treating allergic rhinitis It ought to be considered only when efforts at elimination and treatment of symptoms have proved inadequate

Today, when commercial extracts are still far from perfect and treatment has to continue for several years in order to be effective, cases with only slight symptoms should not be treated with immunotherapy There has to be a reasonable balance between the various problems involved in the treatment, on the one hand, and the degree of severity of the disease, on the other

The grounds for instituting immunotherapy become more important in cases where bronchial hyperreactivity and asthma appear together with the nasal symptoms

GENERAL CONDITIONS

No immunotherapy should be started until the doctor is quite convinced that the allergy diagnosis is correct The allergen planned for treatment must play a decisive part in the disease The number of allergens used simultaneously in the treatment should be limited to a few only

Before starting the treatment a complete analysis of the patient's situation has to be performed Practical prerequisites for completing the treatment must exist This can sometimes be difficult, for instance if the patient is a sailor To choose another example, the indications for immunotherapy are often greater in the case of a farmer with pollinosis than for a clerk

Treatment usually begins during the age span 15 to 30 years Often, in earlier years, the disease has not reached its definitive stage and in higher ages unspecific factors gain in

importance The patient must be given full information about the treatment its practical consequences and effects He or she has to be motivated and accept spending the time needed On the other hand the patient can demand special care at an effectively organized allergy clinic The time at the clinic should be spent after the injection not before

ABSOLUTE CONTRA INDICATIONS

Allergen specific immunotherapy must not be given to patients with other serious immunological diseases Most important are immuno complex diseases for example collagenoses and glomerulonephritis since there is a risk that immunotherapy may activate these diseases

Immunotherapy must not be administered during pregnancy This has been the policy of many allergologists for years based on common caution Our lack of knowledge about possible allergen stimulation of the foetus is reason enough for this A recent publication (Heinonen et al 1977) demonstrates a tendency towards increased frequency of malformations in children borne by mothers who had undergone immunotherapy during pregnancy

In a newly published paper Metzger et al (1978) have made a retrospective study of 121 pregnancies from 90 atopic mothers who had received immunotherapy during pregnancy They were unable to show any increased risk of complications when compared with a control material In the future this result may perhaps lead to a more liberal attitude towards immunotherapy during pregnancy

RELATIVE CONTRA INDICATIONS

It is doubtful whether it is suitable to combine immunotherapy with the treatment of many other diseases such as diabetes malignancies etc Here the decision has to be arrived at mutually by the allergologist and the actual specialist

Atopic eczema urticaria and Quincke oedema may appear during a course of immunotherapy Sometimes earlier symptoms of this kind can be activated In such cases it may be necessary to suspend a treatment already started

Over the last few years allergologists seem to have become increasingly restrictive in the use of immunotherapy especially in cases with hypersensitivity to animals This is probably caused by the new methods of analysing allergen extracts which have sometimes revealed shocking differences in quality and strength in commercial extracts New and better symptomatic drugs have also in many cases reduced the need for immunotherapy

In a strict evaluation of the effect of immunotherapy in asthma and allergic rhinitis it has been shown to be effective in about 80% as compared with 30% for placebo (Juul Sorensen 1976) This is the immediate effect on the disease Immunotherapy might well reduce the risk of allergic rhinitis developing into perennial rhinitis and asthma Some papers give this impression (Johnstone et al 1968 Speer et al 1976) but definite proof is still lacking

The theory that immunotherapy works through stimulation of T-suppressor cells which in the long run make the immuno-competent B-cells disappear provides a good theoretical explanation for a long term effect

It is to be hoped that the new modified and standardized allergen extracts in conjunction with better practical evidence of a long term effect will make it possible for immunotherapy to play a more prominent part in the treatment of allergic rhinitis than is the case today

ZUSAMMENFASSUNG

Bei allergischer Rhinitis sollte eine Immunotherapie zur Anwendung kommen erst wenn sich eine Wirkstoffbeseitigung und eine symptomatische Behandlung als erfolglos erwiesen haben Bei Symptomen einer bronchialen Reizbarkeit oder eines Asthma bronchiale besteht verstärkte Indikation zu immunotherapeutischer Behandlung Eine solche Behandlung setzt jedoch die Erfullung fol

gender Bedingungen voraus Exakte Diagnostik Das für die Behandlung vorgesehene Allergen ist von ausschlaggebender Bedeutung fur das Zustandekommen des Krankheitsbildes Als wichtigste Kontraindikationen für eine Immunotherapie sind andere immunologische Krankheiten sowie Schwangerschaft zu nennen

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A UNIVERSAL WAY TO EVALUATE THE CURVE IN RHINOMANOMETRY

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Abstract A new way to evaluate the curve of nasal air way resistance has been developed With polar coordinates all curves can be described Also tightly closed or wide open noses can be compared with angles The method is of value in clinical as well as in scientific work especially when dealing with statistics

Over the last decade, rhinomanometry has developed from a method of minor importance to a clinically useful tool in parallel with the rapid development of rhinology as a whole

It is necessary to standardize rhinomanometry Most examiners agree that active measurement is best achieved with synchronous registration of pressure difference and flow rate when breathing through the nose *Anterior rhinomanometry is also preferable* Registration in an X Y system is mostly used, and an oscilloscope is best

It has not been possible so far to evaluate the rhinomanometry curve in a uniform way Neither resistance nor conductance can be used at a fixed flow rate or pressure difference on all occasions It is important to be able to make comparisons without limitation

When the nose is tightly closed, the resistance (i e the quotient of pressure-difference/flowrate) cannot be determined at a predetermined flow-rate, as this flow rate is perhaps never reached When on the contrary, the nose is wide open, the conductance (i e the quotient of flow rate/pressure difference) cannot be determined at a fixed pressure difference as this is may be to large

In the ENT Department of the General Hospital in Malmö, clinical rhinomanometry has been in use since 1974, mostly as a diagnostic test prior to nasal surgery Our equipment is easy to handle, using pneumotachygraph and oscilloscope We, like others, have found that active and anterior rhinomanometry is best in clinical work The difficulties mentioned have given rise to problems especially when comparing tightly closed and wide open noses

Analysis of curves from healthy as well as diseased noses, in order to find a mathematical expression applicable under all circumstances was not successful as long as Rohrer's formula (or variants of it) was tried Once more it became obvious that almost closed noses cannot be compared with wide open noses

A new way of evaluating the curve has now been analysed thoroughly When the scale steps of the X- and Y-axes are the same size, three concentric circles around the origin will

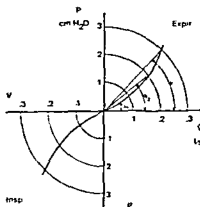


Fig 1 The mathematical treatment of the curve is based on observations at intersections between the curve and the three circles The angle $v(r)$ grows with a greater radius of the circle corresponding to the formula $v(r) = v_0+c \cdot r$

cut the axes at 1 cm H_2O/0.1 l/s, 2 cm H_2O/0.2 l/s and 3 cm H_2O/0.3 l/s, respectively. Angles are determined between the curve and the X axis from three intersections between curve and circles during expiration and three crossing in the same way during inspiration. The six angles are fed into a table calculator and the factors v_0 and c of the formula $v(r) = v_0 + c \cdot r$ can be calculated. v_0 stands for the angle between the curve and X axis where the curve crosses the origin, c is the curvature of the curve, and r is the radius of a circle. The formula has been found suitable for all noses. By adding up the squared differences between the registered and calculated curves, a curve-fitting procedure with a very good result has been possible.

We have not found any limitations with this new way of expressing the curve. Clinical estimation as well as statistical analysis must be easier to manage. v_0 and $v(r)$ are in a group of curves almost normally distributed statistically. Resistance can be expressed as $R = 10 \tan v(r)$, where the factor 10 is the scale difference between the X- and Y-axes.

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HYPOSENSITIZATION IN ALLERGIC SEASONAL RHINITIS AS EVALUATED BY RHINOMANOMETRY

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Abstract Hyposensitization with Allpyral extracts was performed in 38 patients suffering from seasonal allergic rhinitis An objective evaluation of the clinical course was obtained by rhinomanometry After one year of hyposensitization therapy, 55% of the patients (21 out of 38) showed an improvement and after the 3-year follow-up this figure had increased to 76% (19 out of 25 patients continuing with the treatment for this period) For the patients subjective appraisal of the therapy the respective figures were 76 and 92%

Hyposensitization, as a method for treating allergic rhinitis, can be performed with, for example, aqueous and Allpyral extracts The latter possess a number of advantages over aqueous extracts, including (1) less severe side effects—higher doses can therefore be given to highly reactive patients, (2) greater stability—it is therefore unnecessary to reduce the dose on changing to a new sample of the same extract, (3) longer intervals between the injections, and (4) the treatment can be performed entirely in the outpatients' department To judge from the numerous studies that have been published, however, there is no essential difference between the results obtained with aqueous and Allpyral extracts (Gaillard et al, 1963, Caplan & Haynes, 1966 Frankland & Noelpp, 1966, Aschan et al 1978)

Most of the reported results are based on short periods of treatment and a subjective evaluation (Gaillard et al 1963 Frankland & Noelpp, 1966, Evans, 1968 McAllen, 1969), any subjective appraisal is apt to contain a placebo factor (McAllen 1969) Among the objective parameters used are the titre of blocking antibodies and rhinomanometry (Munro-Ashman et al, 1971 Starr & Weinstock, 1971, Fagerberg et al, 1972) In a previous study at this Department in which an evaluation of the therapy was made on the basis of the rhinomanometric technique after one year s hyposensitization, there was no appreciable difference in the results obtained for aqueous and Allpyral extract groups (Aschan et al, 1978)

This paper reports the results obtained with Allpyral extracts over a 3 year period

MATERIAL AND METHODS

The series consisted of 38 patients with allergic seasonal rhinitis (Table I) Hyposensitization treatment was introduced between 1973 and 1975 The diagnostic battery comprised a detailed history, intracutaneous tests and challenge tests with aqueous pollen extracts (Vitrum)

The intra nasal provocation tests were performed with the patient isolated in an air-conditioned cubicle The patient was required to sniff 1 0 ml of allergen extract (usually 1 100) into the nose Nasal decongestion therapy was not permitted for at least 3 days prior to the

Table I *Data relating to the patient material and method*

Number of patients	38
Patient age years	11–64 (mean 27)
Number of allergens used	3–17 (mean 8)
Period of hyposensitization months	
Up till first follow-up	10–33 (mean 14)
Up till second follow-up	36–52 (mean 41)

Table II *Allergen extracts chosen for the follow up provocation tests*

	Follow up	
	1 year	3 year
Timothy	11	10
Ox-eye daisy	3	2
Meadow foxtail	1	1
English rye grass	3	
Alder birch & hazel mixed	4	2
Artemisia vulgaris	1	1
June grass	1	1
Wheat pollen	5	3
Rye pollen	2	
Reed	2	1
Dandelion	2	2
Meadow fescue	2	1
Cocks foot	1	1
Total	38	25

provocation test Rhinomanometry by the technique designed by Aschan and co workers (1956) was performed before and 15 minutes after each challenge The air flow through the nose was measured at a pressure gradient of 10 mm H_2O between the nose and the oropharynx The normal air flow through the nose at this pressure gradient is 30–40 litres per min, a lower figure than this is indicative of nasal congestion The pressure gradient was also determined at a flow rate of 20 l/min At lower flow rates than this the rhinomanometric method is not sufficiently accurate

Hyposensitization was performed with various Allpyral pollen extracts, the number used in a particular patient varying from 3 to 17 (average, 8)

The maintenance dose of the extract for all but one of the patients was 1 0 ml at the concentration 10000 PNU, it was administered at intervals of 6–8 weeks, the exceptional patient, who exhibited pronounced side effects, received only 0 5 ml of the extracts, but at the same concentration and intervals

The provocation tests were repeated after 1 year in all 38 patients, and again after 3 years in the 25 patients who had continued with the hyposensitization therapy for the full period On both occasions the intranasal challenge test was performed with the extract that had produced the most pronounced congestion in the pre hyposensitization test (Table II) All the provocation tests were performed under the above standardized conditions At the 1- and 3-year follow-ups the patients were asked whether, and to what extent, they felt that the treatment had led to an improvement The reasons for 13 patients (including 7 of the 21 patients with objective improvement at the 1-year follow up) dropping out during the last 2 years of the study included change of address, pregnancy, termination of the treatment owing to troublesome side effects, and, in a few cases of unknown reasons

RESULTS

Nasal congestion was considered to exist if the air flow through the nose was reduced by at least 20% and there was also an increase in the pressure gradient after the challenge test In some patients there was such complete obstruction that no values were measurable

At the 1-year follow-up of the Allpyral treatment group a substantial rhinomanometric improvement was found in 21 of the 38 patients (55%) In the remaining 17 patients (45%) there was only a slight improvement, if any

At the 3 year follow-up there was a substantial rhinomanometric improvement in 19 of the 25 patients (76%) continuing the treatment for the full 3 years The remaining 6 patients (24%) showed at best a slight improvement

A subjective improvement was declared by 76% of the patients (29 out of 38) after one year's treatment and by 92% (23 out of 25) after 3 years

DISCUSSION

For any appraisal of hyposensitization therapy in allergic rhinitis to be of practical value it must be based on an objective criterion, as well as on the patient's own evaluation This

is particularly important in the case of this form of treatment, as it is apt to include a placebo factor which may account for as much as 30% of the professed improvement. Rhinomanometry, an objective method for determining the effect of hyposensitization, is easily performed in clinical practice and possesses a high reproducibility. Moreover, it enables a comparison of the mucosal reactions to be made at intervals during the period of hyposensitization.

The proportions of patients reporting a subjective improvement after 1 and 3 years, namely, 76 and 92%, respectively, are similar to those given for other studies based on a subjective evaluation of each symptom recorded in the patients' record cards. From the increase in the proportion of patients achieving an objective improvement—from about one-half to three-quarters over the last 2 years of the 3-year period of the study, it may be deemed worthwhile to persist with the treatment for a longer period than one year. The inconvenience to the patient that this prolongation of the treatment may incur is to some extent ameliorated by the advantages that Allpyral possesses over aqueous extracts, including a longer interval between maintenance doses, treatment entirely in the outpatients' department, and less severe side effects.

ZUSAMMENFASSUNG

Desensibilisierung mit Allpyral Extrakten wurde bei 38 Patienten, die an saisonbedingter, allergischer Rhinitis litten, durchgeführt Die objektive Auswertung des Klinischen Verlaufs wurde mittels Rhinomanometrie erhalten Nach der Behandlungsdauer von einem Jahr wiesen 55 Prozent der Patienten (21 von 38) eine Besserung auf und nach drei Jahren hatte sich dieser Anteil auf 76 Prozent erhoht (19 von 25 Patienten, die in dieser Zeitspanne die Behandlung fortgesetzt hatten) Nach der subjektiven Behandlungsbeurteilung der Patienten war die vergleichende Prozentzahl 76 bzw 92

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PERORAL CHROMONES

A New Way to Treat Allergic Rhinitis?

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Abstract Disodium chromoglycate (DSCG) is well documented in the topical treatment of allergic rhinitis Its use does not lead to any major side effects FPL 57579 is a new chromone compound which is well absorbed after oral administration In a clinical trial patients with allergic rhinitis underwent a nasal challenge before and after ingestion of FPL 57579 The effect on nasal airway resistance (NAR) was determined by rhinomanometry In all cases there was a smaller increase in NAR after the intake of FPL 57579

The ability of disodium chromoglycate (DSCG) to block allergic responses in the nasal airways is well documented (Taylor & Shivalkar, 1971, Backman et al , 1971) when applied locally in the nose either as a spray or as a powder The advantage of DSCG compared with antihistamines and corticosteroids, for example, is that it has no adverse side effects The one disadvantage of DSCG is that it is inconvenient for the patient to administer and that only limited mucosal areas can be reached directly by the substance

The aim of this investigation was to study the effects of a *peroral* chromone on allergic rhinitis If a beneficial effect could be demonstrated, peroral chromones might prove an alternative to the systemic use of antihistamines and steroids and yet preserve the atoxicity of DSCG

MATERIAL AND METHODS

FPL 57579 is a new chromone drug, which is well absorbed after oral administration (Fisons Ltd , 1977) An open pilot trial was designed to study the efficacy of the compound in preventing the increase in nasal airway resistance which follows nasal antigen challenge in allergic patients

The toxicity, metabolism and antiallergic properties of the compound have been studied for several years in the laboratory of Fisons Ltd , Pharmaceutical Division, England in vitro, in animals and in human subjects as well (Fisons Ltd , 1977) In the present trial FPL 57579 was given as a single dose of 24 mg orally

Five healthy adults took part in the investigation One patient was tested twice and was challenged with two different concentrations of the pellen extract

The diagnosis of allergic rhinitis was confirmed by anamnesis and cutaneous prick test The trial was performed during the winter months to avoid spontaneous challenge by pollen in the air At the first visit the patient was challenged with an individually adjusted amount of a fresh, water-soluble pollen extract solution (Vitrum) The extract was dripped on the inferior nasal turbinates The

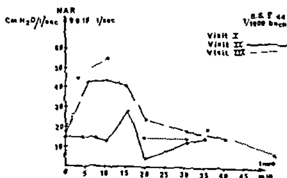


Fig 1 Typical curves for 4 out of 6 cases showing the difference in NAR between visit I and visit III (see text)

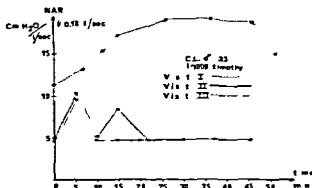


Fig 2 Typical curves for 2 out of 6 cases showing the difference in NAR between visit I and visit III (see text)

effect on the nasal airway resistance (NAR) was recorded with a Rhinomanometer (NR 1, Mercury Electronics Ltd, Scotland + X–Y writer MINIGOR, type RE 501, Goerz Electro, England) Nasal discharge and the number of sneezings were estimated by both the patient and the doctor

At the second visit, exactly the same procedure was repeated 2–3 hours after the administration of 24 mg FPL 57579 per os At the third visit exactly the same procedure as at the first was repeated Haemoglobin, leukocytes, .. enzymes and bilirubin in serum were ted before and after the trial

RESULTS

In all 6 cases a substantial increase in NAR was recorded after the initial challenge (visit I) Each patient had a considerably weaker mucosal reaction after the intake of FPL 57579 (visit II) In 4 patients the stronger reaction at visit I could be reproduced at visit III (Fig 1), but in 2 cases this could not be demonstrated (Fig 2)

The challenges were performed at time 0 V' is the airflow through the nose at which NAR was determined The only side effect recorded was slight nausea in one patient about one hour after the administration of the substance Laboratory findings on blood samples were normal before and after the trial in all patients

Subjective scoring of nasal discharge and sneezing

All patients developed their seasonal symptoms at visit I and visit III There was less nasal discharge as well as sneezing at the post administration with the substance (visit II) There was close correlation between the estimations made by the patients and the authors

DISCUSSION

As mentioned above, in 4 cases out of 6 the rhinomanometric recordings showed a substantially smaller increase in NAR upon challenge after administration of FPL 57579 (Fig 1) In these 4 cases it was possible to reproduce the mucosal reaction from visit I after 4 weeks (visit III)

In 2 cases there were not the same increase of NAR at visit III as at visit I (Fig 2) Unfortunately more than 8 weeks had elapsed between visit I and visit III and it is possible that the extracts had become inactivate (Hjort, 1957)

This study shows that FPL 57579 reduces the allergic mucosal reaction expressed as an increase in NAR when sensitized patients are challenged with pollen extract in the nose The side effects arising were minimal—at least when the substance was administered as a single dose

Further clinical investigations are necessary to evaluate whether peroral chromones constitute a new way of treating allergic rhinitis The optimal dose and possible side effects during long term treatment are still unknown as also are the possible effects of the more general blocking of mast cells that this type of treatment may involve

ZUSAMMENFASSUNG

DSCG hat einen guten dokumentierten Effekt in der lokalen Behandlung von allergischer Rhinitis und die Substanz hat keine großene Nebenwirkungen FPL 57579 ist eine neue Chromonvereinigung, die sich gut absorbiert nach per os Administration In einer klinischen Untersuchung wurde Patienten mit allergischer Rhinitis vor und

nach der Behandlung mit FPL 57579 in der Nase mit Pollen provioziert Der Effekt auf den Nasenwiderstand wurde mit Rhinomanometrie studiert In allen Fällen wurde eine geringere Steigerung in dem Nasenwiderstand nach der Behandlung mit FPL 57579 registriert

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DISODIUM CHROMOGLYCATE THERAPY IN PERENNIAL RHINITIS

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Abstract DSCG was tested on 38 patients with perennial rhinitis in the form of a nasal spray (2%) in a double blind cross-over trial. Few of the investigated parameters in the whole material showed any significant preference of DSCG. A marked drug order effect was shown, in the group receiving placebo first, most of the clinical parameters assessed both by the clinician and the patient were better for DSCG. A therapeutical trial with DSCG is worth trying in perennial rhinitis.

Disodium chromoglycate (DSCG) was first used as an antiallergic drug in asthma (Altounyan, 1967), since when it has gained a wide use in allergic rhinitis as well. In both of these conditions its primary mechanism of action is to prevent the release of the chemical mediators from the mast cells (Brain et al., 1974, Orr & Cox, 1969).

Recently, DSCG has also been used on patients with perennial rhinitis, in the form of both insufflated powder and sprayed aqueous solution. The drug is supposed to prevent the mast cell degranulation also when non-immunological factors are important (Holopainen et al., 1975).

MATERIAL AND METHODS

The investigation was carried out on 38 patients who had had perennial rhinitis of such severity as to require treatment for at least one year. Patients were included irrespective of the aetiology of their disease which was thought to be allergic for 27 and intrinsic for 11 patients.

The period of investigation lasted for 8 weeks. During this time every patient was receiving the active drug for 4 weeks and placebo for another 4 weeks. The dose was 0.13 ml of 2% aqueous solution of DSCG (or placebo respectively) six times a day to each nostril. The sequence of the treatments was randomized and the treatments were given on a double-blind basis.

Nasal smears for evaluation of the cytologic picture were taken at the outset, after 4 weeks and after 8 weeks. At the same points of the study, the clinician recorded his opinion on a four-point scale according to the severity of the symptoms of nasal blockage, nasal running and sneezing, together with his estimate of the patency of each nostril.

Throughout the period of investigation, the patients kept a daily record of the severity of their symptoms of nasal blockage, nasal running, nasal irritation, sneezing and any additional treatment used. All side effects were recorded at the end of each half of the study. At the end of the investigation the treatment preference was asked.

RESULTS

The only significant differences in the clinician's estimates (tested by the Wilcoxon matched pairs signed rank test) in favour of DSCG were seen in parameters of running

Table I *Patient's preference*

Drug order group	DSCG	Placebo	Neither	2 tail binomial probability
DSCG – Placebo	9	7	2	0.804
Placebo – DSCG	13	4	3	0.050
All patients	22	11	5	0.082

Table II *Side effects*

Side effect	Number of complaints during treatment	
	DSCG	Placebo
Nasal irritation	8	10
Nausea	2	0
Headache	5	5
Sneezing	0	1
Tiredness	0	1
Paranasal eczema	1	0

(placebo–DSCG group and all patients) and nasal patency (placebo–DSCG group)

In patient's scores there were significant differences in favour of the active drug with respect to sneezing, blockage and running in the patients who received placebo first On the other hand, in the DSCG–placebo group, blockage showed even better figures for placebo No differences were found in the nasal irritation

The nasal smears showed a trend to lower eosinophil scores after the active drug in all patients There were no overall differences in patient preferences but the placebo–DSCG group showed an almost significant DSCG preference (Table I) There were no differences in the use of antihistamines or in the side effects (Table II)

Most of the results in favour of DSCG were found in the group receiving placebo first When studied separately with the Mann Whitney U-test the order effect was found to be significant with respect to nasal patency (clinician s scores) and to nasal blockage, running and sneezing in patient s scores

DISCUSSION

The overall results in our material showed only a slight difference in favour of DSCG This is in agreement with the report of Mygind et al (1972) When using the insufflated powder Warland & Kapstad (1977) found no difference between the active drug and placebo In both of the cited papers the authors consider their dosage too low—it was the same in our study and that of Mygind et al On the other hand many reports in the literature claim better results with DSCG no matter whether the drug was in the form of powder (Holopainen et al , 1972) or solution (Holopainen et al , 1975)

In contrast to the overall results our study showed a difference in favour of DSCG in the group receiving placebo first Separately tested, this order effect was found to be statistically significant This finding is concordant with previous literature (Fagerberg & Zetterstrom, 1975) This order effect must be due to a rather long lasting carry-over effect of DSCG

There was a considerable lowering of the symptom scores even during the placebo period The placebo effect was obviously remarkable in patients who had been treated by various means with only transitory favourable results A new method of treatment, especially a local one, must have had a marked psychological effect Additionally the placebo may have had some real farmacological effect due to the antimicrobial properties of its preservative components

Based mainly on the results in the group receiving placebo before DSCG, we consider the treatment successful and suggest a therapeutical trial of the drug in perennial rhinitis

ZUSAMMENFASSUNG

Doppelblinde uberkreuzende Studien wurden an 38 Pa

Klinikern und Patienten ausgewerteten Parameter zu gunsten von DSCG Es lohnt sich eine Behandlung mit DSCG an Patienten mit perennierender Rhinitis zu probieren

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DSCG EYE DROPS IN ALLERGIC RHINO CONJUNCTIVITIS

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Abstract Thirty patients with allergic rhinoconjunctivitis were treated with eye drops containing 2% DSCG during the pollen season of 1977 Sixteen patients were treated with active substance and 14 with placebo The results show a statistically significant difference in favour of the DSCG drops DSCG in eye drops seems to be a valuable complement to the drugs available today for the treatment of allergic rhinoconjunctivitis

The treatment of vernal rhinoconjunctivitis as well as other allergic diseases is based upon three major principles elimination of allergens, hyposensitization, and use of drugs Pollens are almost impossible to avoid, and hyposensitization is not always effective and is often time consuming in relation to the short pollen season and even sometimes accompanied by untoward reactions The majority of drugs used in allergy treatment have the disadvantage of side effects, e g antihistamines and corticosteroids A great many reports have demonstrated that topically administered disodium chromoglycate (DSCG) has an excellent effect in preventing bronchial asthma and rhinitis caused by pollen allergy (Engstrom & Vejmolova, 1970, Engstrom et al, 1971 Frostad, 1977) No side effects of any importance have so far been reported It would seem logical to assume that DSCG could constitute a safe but effective drug for the topical treatment of allergic conjunctivitis as well

MATERIAL AND METHODS

Thirty healthy adults with confirmed allergic rhinoconjunctivitis caused by pollens have been studied—14 men and 16 women, mean age 29 years The investigation took place during the pollen season May–June 1977 and was a double blind trial The "active" drug was composed of 2% DSCG, 0 01% benzalkonium chloride and 0 4% 2 phenylethanol The *placebo drug had the same composition except* for DSCG One drop was applied in each eye six times a day for 3 weeks The patients were asked to fill in a diary card on which they scored their symptoms (from "0", no symptoms at all, to "4", very severe symptoms) Variations in the weather were also noted A thorough medical examination of the patient was made before the start of the study and immediately after the test period The symptom scoring by the investigators was used simultaneously with those of the patients

RESULTS

The results are based on a statistical calculation of the symptom scores Success means that the score reached "0" or decreased by at least 50% One patient was excluded from the final assessment

	Active	Placebo
Success	13	6
Failure	1	5
Not sure	1	3

The DSCG drops were successful in 87%, while the placebo drops were reported successful in 43% The difference is significant, $p<0.05$

Side effects

Seven patients in the active group and 6 in the placebo group reported a mild and very transi

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ent stinging in the eyes when applying the drops Only one patient stopped the treatment because of this No other side effects were reported

DISCUSSION

The results show good agreement with those from treatment of allergic rhinitis with DSCG, usually a success rate of 70–80% (Engstrom et al , 1971, Easty et al , 1972, Frostad, 1977, Kazdan et al , 1976, Wardell et al , 1977)

No less than 43% of the patients in the placebo group reported success, which seems to be within the 'normal range' of placebo effects

CONCLUSION

Eye drops containing 2% DSCG constitute an effective remedy almost entirely lacking in side effects, for treatment of allergic conjunctivitis It is of great importance that atoxic and effective pharmaceutical agents for topical administration to the allergic mucous membranes should be produced and tested DSCG in eye drops tested in this study is an example of such an agent that may reduce the need for hyposensitization, systemic antihistamines and corticosteroids in vernal rhinoconjunctivitis

ZUSAMMENFASSUNG

Dreissig Patienten mit allergischer Rhinoconjunctivitis sind wahrend der Pollenzeit 1977 mit Augentropfen die 2% DSCG enthalten behandelt worden Sechzehn Patienten sind mit aktiver Substanz behandelt worden und vierzehn mit Placebo Die Resultate zeigen einen signifikanten Unterschied zugunsten der DSCG Tropfen DSCG in Augentropfen scheint eine wertvolle Erganzung zu den Heilmitteln die wir heute in der Behandlung von allergischer Rhinoconjunctivitis haben zu sein

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NASAL METHACHOLINE PROVOCATION AND IPRATROPIUM THERAPY OF PERENNIAL RHINITIS

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A hyperreactive mucosa, which reacts more strongly than normally to unspecific stimuli, is a common feature of two entirely different diseases, namely perennial rhinitis and asthma (Connell, 1970, Borum, 1978 I) However, while this hyperreactivity can be measured in asthma patients by means of methacholine or histamine challenge of the bronchi (United States National Tuberculosis Association, 1967, Spektor & Farr, 1975), no test exists today for measuring the hyperactivity of the nasal mucosa Therefore, it would be rational to attempt to devise a test, similar to the methacholine challenge of the bronchi, for perennial rhinitis This would permit the patients being allocated to various sub-groups within the disease, and further would aid the diagnosis, as at present this is based mainly on the patients' own description of their symptoms Such a test would also allow the efficacy of various treatments to be compared, using truly scientific parameters (Spektor & Farr, 1975) Possibly it could also provide valuable pathophysiological information

The parasympathetic nervous system innervates the glands of the nasal mucosa (Cauna, Cauna & Hinderer, 1972) Many research workers consider that the hyperreactivity of perennial rhinitis is caused by an imbalance of the autonom nervous system, and that there is a parasympathetic predominance (Fowler, 1949, Golding-Wood, 1961, Krajina, Harvey & Ogura, 1970) The glands of the upper airways contain few, if any, histamine receptors, and as the symptoms of allergic perennial rhinitis are caused by the release of biochemical mediators, in particular histamine, it is presumed that histamine induced hypersecretion takes place via the epithelial irritant receptors, together with reflex activity in the secretory (parasympathetic) nerves (Okuda, 1977) Hyperreactivity within the nervous system may be the cause of the increasing reactivity seen throughout the season in patients suffering from seasonal rhinitis (Connell, 1970) This has been supported by Okuda, who produced a blockade of the mucosal irritant receptors, by spraying the nose with leostesin, prior to allergen provocation, thereby preventing the occurrence of allergic symptoms Theoretically, stimulation of the nasal mucosa, by means of a parasympathomimetic compound such as methacholine, in patients with perennial rhinitis should produce larger amounts of secretion than in normal subjects, and therefore an obvious choice of treatment would be the use of a compound having effects similar to atropine

The present investigation was designed to develope a simple test of nasal reactivity This was successful, inasmuch as it was found that methacholine was well-suited for the purpose Further to determine whether the application of the topically active parasympatholytic drug, Ipratropium (Atrovent®, Boehringer-Ingelheim) to normal subjects would be able to block methacholine induced hypersecretion Atrovent® is supplied for this purpose as an aerosol In addition, to ascertain whether the same drug was able to reduce the hypersecre-



Fig 1 Nasal secretory response to methacholine challenge in 20 healthy subjects Relationship to methacholine dosage and time

tion (spontaneous) of patients suffering from perennial rhinitis It was found that the compound was able to do this, without causing any local or systemic side effect, and that it was so successful that the compound is now used in our department in the treatment of perennial rhinitis with hypersecretion as a dominant symptom

Nasal methacholine challenge

Twenty healthy experimental subjects and 30 patients suffering from perennial rhinitis were included in this experiment (Borum 1978 I) In order to be admitted to the test the patients had to fulfil the following criterion two of the following symptoms for at least one hour almost every day attacks of sneezing colourless rhinorrhoea or nasal stenosis due to swelling of the nasal mucosa

By dissolving 3 6 12 24 and 48 mg of methacholine, respectively in 0 4 ml of distilled water, a titration series was produced Four tenths of a ml of the solution were sprayed into the nose to produce the challenge, employing a DeVilbiss nebulizer No 15 Thereafter the subject bent his/her heads forward and remained in that position for the following 15 minutes The secretion was meanwhile collected and the number of sneezes and drops noted The funnel led directly to a syringe so that the volume of secretion could be measured directly The challenges were repeated in intervals of 30 minutes, using increasing concentrations of the compound from 3 to 48 mg However, as there is a risk of bronchospasms with higher doses than 6 mg/0 4 ml in patients with perennial rhinitis, that concentration was the only one used in such patients

Within a few minutes of the challenge in both normal subjects and patients a watery secretion began to drip from the nose (Fig 1) It was found that the dose–response relation ship was linear But women produced considerably more secretion than men, with standard doses ($P<0\ 001$)

The patients suffering from perennial rhinitis produced more secretion than the test subjects, with the standard dose (6 mg) (Fig 2 $P<0\ 001$)

Ipratropium (2 puffs, 40 μg into each nostril) given to normal subjects 30 minutes prior to the challenge, resulted in a parallel displacement of the log dosage curve This indicated a competitive inhibition of the cholinergic receptors (Fig 3)

A S D of 25% of the mean was obtained when the challenge was repeated at a later date, indicating that the reproducibility of the test was reasonable No effects on the blood

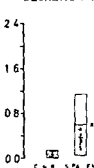


Fig 2 Resulting secretion (mean ± S D) after nasal challenge with 6 mg methacholine in 20 healthy subjects and 30 patients with perennial rhinitis ($P<0\ 001$)

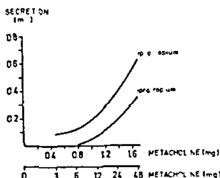


Fig 3 The log dose-response relationship between intranasal nebulized methacholine and the resulting amount of secretion in 15 healthy subjects with and without Ipratropium pre treatment The drug brought about a significant reduction in secretion ($P<0.001$) and a parallel displacement of the log dose-response curve indicating a competitive inhibition

pressure or pulse were noted after the use of either methacholine or Ipratropium

The present investigation thus shows that it is possible to produce measurable amounts of nasal secretion by methacholine challenge both in normal subjects and in patients with perennial rhinitis It is noteworthy that the patients with perennial rhinitis reacted more violently to the challenge than did the normal test subjects The test devised here is simple to carry out, causes no great discomfort to the patients and requires no expensive equipment

Intranasal Ipratropium

Ipratropium (Atrovent®, Boehringer Ingelheim) is a topical parasympatholytic drug, supplied in aerosol form and used for the treatment of bronchoconstrictive disease as a broncho-dilatator (Poppius & Salorinne, 1973) When employed in therapeutic doses, it has not system effect (Wieser & Koenigshofer 1975) As mentioned earlier, the hypersecretion of patients with perennial rhinitis should be inhibited by a parasympatholytic compound In all probability the effect of antihistamines and tricyclic antidepressants are also based—at least in non allergic perennial rhinitis—on their parasympatholytic activity

In 15 healthy subjects with hypersecretion brought about as described earlier, an attempt was made to block this hypersecretion by the use of Ipratropium (Borum, 1978 II), the object being to obtain an answer to the following questions before carrying out a more detailed and comprehensive clinical trial of the compound

(1) Does Ipratropium inhibit the receptors of the glands when applied intranasally by means of an aerosol?

(2) What is the required dose?

(3) If such a block does occur, for how long is it effective?

(4) What local or systemic side-effects occur, if any?

In a double blind cross over trial Ipratropium or placebo were sprayed into each nostril 30 minutes before a methacholine challenge Fig 4 shows that the Ipratropium was able to reduce significantly the amount of secretion induced by methacholine Ten patients suffering from perennial rhinitis were similarly treated in order to ascertain whether the compound was also effective in such cases Fig 5 shows that Ipratropium caused a significant reduction in the secretion in these patients also

Normal test subjects were pretreated with Ipratropium 5 and 30 minutes, 1, 2, 4, 6, 8 12 and 18 hours, before a methacholine chal

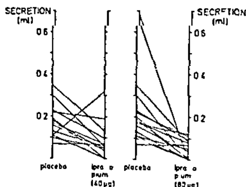


Fig 4 Inhibition of methacholine induced nasal secretion by Ipratropium in 15 healthy subjects *Left* One puff (20 µg) of Ipratropium in each nostril before methacholine challenge reduced the secretion significantly ($P<0.001$) *Right* Two puffs (40 µg) in each nostril had an even greater effect but the difference between the two dosages was not significant ($P<0.20$)

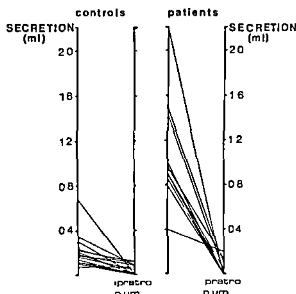


Fig 5 Inhibition of methacholine induced nasal secretion by 80 μg of Ipratropium in 15 healthy subjects and 10 patients with perennial rhinitis The patients produced far more secretion on the same dosage of methacholine (6 mg) ($P<0\ 001$) Two puffs of Ipratropium into each nostril reduced the secretory response significantly ($P<0\ 001$) not only in the healthy subjects but also in the patients

lenge, in order to determine the dosage–time relationship An interval of 24 hours was allowed to elapse between the tests The application of Ipratropium caused a 30% reduction in the secretion as early as 5 minutes after the challenge using methacholine The effect was maximal during the period 4 to 6 hours, after which it gradually declined Thus this investigation showed that the cholinergic receptors could be effectively blocked by the use of 40 μg (2 puffs) of Ipratropium 3–4 times per day, applied to each nostril

A full scale clinical trial could be carried out to ascertain the effects of Ipratropium after these laboratory tests as an idea could then be obtained of the dosage required and the necessary time interval between them

Ipratropium treatment of perennial rhinitis

The investigation included 20 patients with perennial rhinitis, all of whom had hypersecretion as the main symptom of the disease Nearly all of the patients had tried various other drugs for relief of their complaint without any very convincing results The trial was carried out using a double-blind cross over technique (Borum, Schultz Larsen & Mygind, 1978 III)

The patients completed a score card one week before entering the trial and after having discontinued any medication for at least one month They were treated randomly for two periods of 14 days with Ipratropium or placebo Two puffs being given into each nostril four times per day The code of the investigation was not broken until all the results had been evaluated

Fourteen of the 20 patients preferred the active compound, 3 the placebo, while 3 others had no preference ($P<0\ 006$) The effects of the drug were so convincing to the 14 patients that they were willing to take part in a long term trial of the compound Ipratropium had its effect on the hypersecretion, this was the symptom employed when selecting those to take part in the trial On the other hand the drug had no effect on sneezing or nasal stenosis

Conclusion

Intranasal application of methacholine produced in the present investigation hypersecretion both in normal subjects and in patients with perennial rhinitis This test permitted the differentiation between normal subjects and patients, and further, it was noted that women produced significantly greater amounts of secretion than men The application of methacholine was found to be a suitable test for determining the dosage, duration of action etc of a new parasympatholytic drug (Ipratropium, Atrovent®, Boehringer Ingelheim) The controlled clinical investigation following the development of this test showed that Ipratropium was effective in counteracting spontaneous hypersecretion in the majority of patients having this symptom as the main component of perennial rhinitis The study suggests that in future Ipratropium will take its place in the treatment of rhinorrhoea

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PAROSMIA

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Abstract Changes in the sense of smell may be quantitative or qualitative The latter are called parosmia Various forms of central and peripheral parosmia are described The treatment is mentioned

Changes in the sense of smell may be of quantitative or qualitative origin, or possibly even a combination of both

Quantitative changes may manifest themselves partly as hyperosmia and partly as hyposmia or anosmia The latter two types are by far the more common

Qualitative changes have been designated by Hoffman (1926) as parosmia

Parosmia can be divided into two types the peripheral and the central In some cases parosmia is of a mixed peripheral and central type

I *Central Type of Parosmia*

(*a*) *Illusions of smell* may occur premenstrually, in pregnancy, in hemicranic patients and in neurotic patients

(*b*) *Olfactory hallucinations* may occur in psychotic patients, especially schizophrenics

(*c*) *Uncinate fits* (Jackson & Beevor, 1890), i e attacks of sensations of smell, possibly accompanied by other phenomena of temporal irritation These sensations are elicited by irritative processes in the anterior part of the temporal lobe, the uncus

II *Peripheral Type of Parosmia*

(*a*) *Cacosmia* i e a periodical or constant bad smell due to abnormalities of the nose, sinuses and pharynx, such as sinusitis, atrophic rhinitis, ozaena and tonsillitis

(*b*) *Essential parosmia* is taken to mean a condition in which stimulation of the sense of smell does not result in an adequate olfactory impression, but in a perverted sensation, nearly always of a foul odour

(1) *After intoxication*, systemic (e g streptomycin) or after topical application to the nose (e g tyrothricin) Solvents have been reported to cause parosmia and hyposmia (Emmett, 1975)

(2) *Infectious diseases*, especially influenza may be followed by long-continued essential parosmia This has been reported especially by doctors, who have had this experience

(3) *Cranial injuries* may be followed by long lasting parosmia, even in patients whose sense of smell is greatly impaired In Sumner's (1964) series of 1 167 patients with cranial trauma, 0 34% suffered from parosmia independent of the severity of the cranial trauma 7 5% of the patients had anosmia dependent on the severity of the cranial trauma

(4) *Cause unknown* Most cases belong to this group

Treatment of essential parosmia is difficult The best treament in our experience is blocking of the olfactory region with cocaine with which most of the patients were curred In this treatment the patient lies supine, with his head bent far back, so that the external porus acusticus and the chin are in the same vertical plane In this position the olfactory region is undermost, and two drops of 10% cocaine hydrochloride in physiological saline instilled into the nostrils will run down into the olfactory region (Zilstorff, 1965) The patient lies in this position for about 30 sec Another treatment is given each day for the next 2–4 days

A few patients require 2–4 series of treatment weeks to months later because of relapse

In a few patients not cured by this therapy we used a masking effect of menthol ointment applied in the nose, especially before eating Two cases are mentioned

(1) A 66-year old man, who complained of a parosmia for 6 years after a severe cranial trauma His olfactory thresholds (M P O, Zilstorff 1957) were normal and the neurological and neuro radiological examinations were also normal He was cured after a few treatments with cocaine

(2) A 30-year old man, who had had parosmia of unknown origin for the last 8 years both for food and spontaneously Neurological and neuro radiological examinations were normal and so were his olfactory thresholds After the first treatment he improved so much that he could take up his scientific work again which he had stopped for about a year It was necessary to give him several more series of cocaine treatment over the next 3 years For the last 5 years he has been without symptoms

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EARLY, ACTIVE DIAGNOSIS OF ACOUSTIC NEUROMAS

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Abstract Since the introduction of the translabyrinthine approach in the treatment of acoustic neuromas in Denmark 47 patients have been operated on the series containing 2% small 60% medium and 38% large tumours This distribution differs significantly from previously published tumour series where the large tumours dominated The paper describes the various diagnostic procedures applied in the present active search for acoustic neuromas and the reduction in tumour size is ascribed to information on the improved surgical results obtained by the translabyrinthine approach

In the early days of acoustic neuroma surgery it became quite evident that smaller tumours were far more amenable to radical removal than were large tumours (Cushing 1917, Dandy, 1925) In 1976 the translabyrinthine approach was introduced in Denmark, and at same time a more intensive search for [s]maller tumours was initiated This paper describes the order of the various diagnostic procedures applied to the patients by the various diagnosticians from the general practitioner, the ENT specialist without hospital affiliation, to the various hospital ENT departments and neuro radiological departments, where the final diagnosis is either confirmed or disproved The paper also presents the tumour distribution obtained by this active search, while the results of the translabyrinthine surgical treatment is presented in a subsequent paper (Tos & Thomsen 1979)

MATERIAL AND METHODS

In a previously published material from 1957 to 1972 (Thomsen, 1976) concerning 125 acoustic neuromas, only 15% could be classified as medium sized, while the rest (85%) were large tumours From 1972 to 1976, 27 patients with acoustic neuromas were operated on suboccipitally at Rigshospitalet, with 30% medium sized tumours, the remaining 70% being classified as large (Table I) In contrast, 47 patients have been operated via the translabyrinthine approach between 1976 and 1978, with one small tumour (2%), 28 medium (60%) and only 18 (38%) large tumours (Table II)

DISCUSSION

The initial symptom of an acoustic neuroma is in the vast majority of patients, unilateral hearing impairment and/or unilateral tinnitus However, these symptoms are often neglected by the patients, and frequently they do not seek medical advice until they eventually are "lucky" enough to get their first vertiginous attack It may be advisable, through information in the medical columns of newspapers and magazines, to alert the patient and make him aware of the possible significance of these

Table I *Acoustic neuromas, 1957–72 in 125 patients and 1972–76 in 27 patients*

1957–72		
Small	0	0%
Medium	20	15%
Large	105	85%
1972–76		
Small	0	0%
Medium	8	30%
Large	19	70%

Table II *Tumour distribution with active search for acoustic neuromas 1976–78 in 47 patients*

Small	1	2%
Medium	28	60%
Large	18	38%

symptoms, and induce him to consult a physician

The general practitioner is first in line, and he should know the possible cause of the symptoms presented by the patient He may perform tuning fork tests in order to separate conductive from sensori neural hearing impairment, but he should confine his treatment to removal of ear wax in the ear in question With persistence of the symptoms after this procedure the patient should be referred to an ENT specialist The ENT specialist is competent to establish the definitive diagnosis of sensori neural hearing impairment He should also in these cases obtain a complete oto neurological evaluation and refer the patient to a radiologist for tomography of the internal acoustic meatus In cases of unequivocal signs of cochlear type of involvement, combined with normal caloric function as well as normal tomography, referral to an ENT department is not indicated The ENT specialist who has no hospital affiliations may check the patient, e g once a year including tomography of the petrous bone, and this should be done for several years If any change in the audiological configurations occurs, if the caloric response deteriorates and/or there are signs of destruction of the internal acoustic meatus at tomography, the patient should be referred to an ENT department for further evaluation The ENT specialist should also refer such patients to the hospital when the caloric function and/or tomography is abnormal, even if the pure tone audiogram is unaffected Also any finding of involvement of the facial, intermedius or trigeminal nerves, or presence of cerebellar dysfunction or a defective optokinetic nystagmus should prompt referral to a hospital ENT department It must be emphasized that there is no need for lumbar puncture and spinal fluid protein examination, since this examination is of no value (Thomsen et al , 1978)

In the ENT department the first diagnostic steps are centered around 1) Conventional audiological tests, 2) Differential-caloric testing, 3) Audiological brain stem examination, 4) Tomography of the internal acoustic meatus If *two* of these diagnostic procedures indicate suspicion of an angle tumour the patients should be submitted to neuro radiological examination The presence of facial, intermedius, trigeminal, cerebellar or optokinetic symptoms also indicates a neuro-radiological examination, if they furthermore are accompanied by just *one* of the above mentioned symptoms In equivocal cases, where the patients have not obtained enough points for neuro radiological procedures, as described by Thomsen et al (1977), the patient should be followed at regular intervals, and the tests repeated e g once a year

With regard to the auditory brainstem responses, as described by Thomsen et al (1978) we feel that it is such an important test, with the advantage of being a non invasive procedure, that it should be performed in dubious cases even if it implies that the patients have to be referred from one ENT department to another department having this equipment The reason is that the alternative to this test will often be the invasive Pantopaque cisternography, which is of course only indicated if sufficient suscion of angle tumour exists, and if computer tomography is available, only after this examination

Auditory brainstem responses can be combined with electrocochleography, giving even more information, but the combination is more time consuming (Elberling et al , 1978)

Concerning Pantopaque cisternography, there is no doubt that in small- and medium-sized angle tumours it is the final and most conclusive neuro radiological procedure However, the authors feel that the performance of Pantopaque cisternography should be

confined to neuro-radiological departments having a specialized interest in these problems, since in proper hands it is a simple and conclusive study but it tends to become a useless and confusing examination in the hands of the inexperienced.

A continuous education of all physicians who may possibly become involved in the diagnosis of acoustic neuromas is necessary. Through postgraduate courses and medical papers they should be informed about the improved possibilities for detecting these tumours in the early stages Just as important is information about the progress in surgical results of the treatment. Translabyrinthine surgery in Denmark has been a strong stimulus for a more active search, and considerably more (especially smaller) tumours are being diagnosed today than 2 years ago (Tos & Thomsen, 1979).

ZUSAMMENFASSUNG

Seit der Einführung der translabyrinthären Operationsmethode in der Behandlung der Acusticusneurinomen in Dänemark sind 47 Patienten operiert worden Dabei waren die Tumoren in 2% der Fälle klein, in 60% mittel und in 38% groß Diese Verteilung unterscheidet sich signifikant von den früheren Veröffentlichungen, wo die großen Tumoren vorherrschend waren Die Arbeit beschreibt die verschiedenen diagnostischen Vorgänge, die jetzt in der aktiven Such nach Acusticusneurinomen verwendet werden Die Reduktion der Tumorgroße ist hauptsächlich auf die Berichte uber die verbesserten Ergebnisse bei der neuen Operationsmethod zuruckzuführen

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TRANSLABYRINTHINE SURGERY OF ACOUSTIC NEURINOMA

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Abstract In translabyrinthine and translabyrinthine suboccipital removal of 47 acoustic tumours the mortality rate was 2 1% among 18 large tumours 5 6% The facial nerve was preserved in 89% and 80% had normal facial nerve function 98% of the tumours were removed *in toto* including 17% where the tumour remnant was removed by the suboccipital approach one week after the translabyrinthine operation

After the pioneer work done by House (1964) large series of acoustic neurinomas removed by the translabyrinthine approach have been reported (House 1968, Clemis, 1971, Montgomery, 1973, Maddox, 1977 Glasscock et al, 1978, Morrison 1978 and others) A common feature of all these materials is a high percentage of patients with preserved function of the facial nerve, a low mortality, and good post operative condition

The results of translabyrinthine surgery of acoustic neurinomas in Denmark on 47 patients in the course of the past 2½ years are presented here

MATERIAL AND METHODS

The material comprises 47 patients with acoustic neurinoma subjected to operation during the past 2½ years Mean age was 49 (range 18–73) years and 12 patients were over 60 According to the classification of House (1968) one patient had a small intrameatal tumour 28 had medium sized, and 18 had large tumours Among the latter the extrameatal part of 15 tumours exceeded 4 cm in diameter

All patients were primarily treated by translabyrinthine operation (House, 1964) In 8 patients with large tumours the tumour could not be removed *in toto* but its size was reduced and the facial nerve was freed and marked with Silastic (Montogomery, 1973) One week later, the tumour remnant was removed via the suboccipital approach

RESULTS

Mortality One patient with a large tumour died one month after the suboccipital operation (Thomsen et al, 1977) After the translabyrinthine operation there were no deaths The total mortality was 2 1%, among large tumours 5 6%

Preservation of facial nerve Two patients had preoperative facial palsy In one of them a medium sized tumour extended right up to the geniculate ganglion, destroying the Fa[illegible] lopian canal in which no nerve fibres could [illegible] identified In the other patient, who had a [illegible] tumour, the facial nerve could be freed [illegible] meatus and on a level with the porus [illegible]

Table I *Preservation of the faci[illegible] postoperative normal functio[illegible] nerve in 45 patients with ac[illegible]*

Approach size	[illegible]
Translabyrinthine	
Small (1)	
Medium (27)	
Large (9)	
Translab + suboccip[illegible]	
Large (7)	
Total (45)	



Fig 1 EMI scanning of the biggest acoustic tumour in the material totally removed in two stages

internus, but during the suboccipital operation in could not be followed in its most proximal course Indeed, both had postoperative palsy and are not included in the calculations of facial nerve function which are based upon 45 patients

Among the 38 patients treated exclusively by translabyrinthine operation, the facial nerve could be preserved in 36 (95%) In 2 cases the nerve was torn by unfortunate accident in a case of a medium sized tumour the entire facial nerve had been freed, and it was intact Thereafter part of the nerve, on a level with the porus acusticus was covered with surgical putty After the putty was removed, the nerve had been torn However, via the translabyrinthine approach it could be sutured with two silk sutures a procedure which Drake (1967) considered impossible In the case of the other tumour which was a large one, the nerve was freed in the internal meatus and on a level with the porus After intracapsular reduction of the tumour the nerve was seen to be intact at the entrance to the brain stem, but later it got torn at this site Both examples show that the facial nerve may be injured even after it has been freed

Among patients with large tumours we were able to preserve the facial nerve in 77%, with in the total series, in 89% (Table I)

A normal function of the facial nerve has so far been achieved in 80% of the patients and in this respect there is a great difference between the translabyrinthine and the translabyrinthine suboccipital removal (Table I) Another 5 patients (11%) have preserved some function of the facial nerve

Total removal 46 tumours (98%) were removed *in toto*, but in 8 (17%) cases this required operation in two stages The only patient whose tumour has not yet been removed is a 68-year old man with bronchial asthma and in a poor cardiovascular condition, who had a 5 cm large tumour We had decided to remove this tumour by the translabyrinthine approach and had already removed the greater part when cerebellar oedema restricted the access to such an extent that it was impossible to get the remainder out On the next day the patient's neurological status was normal and it seems probable that the oedema was due to hypoxia of the cerebellum Owing to the poor cardiovascular status, suboccipital removal of the tumour remnant is hardly indicated but we are following the patient by EMI scanning

Postoperative liquorrhoea Six patients (13%) had postoperative liquorrhoea through the Eustachian tube lasting for a maximum of 10 days, in one somewhat longer This patient had been discharged on the 10th day without liquorrhoea and was feeling perfectly well He started working in his garden, when the liquorrhoea started After 4 weeks the liquorrhoea fistula in the dura of the posterior cranial fossa was closed by a large graft of fascia lata The inserted abdominal fat was vital

Cerebellar affection After the translabyrinthine operation no patient had cerebellar affection or other cranial nerve palsies, except for the eighth

DISCUSSION AND CONCLUSION

By close collaboration with the neurosurgeons, we have succeeded in almost completely centralizing surgery of acoustic neurinoma in Denmark. With a total population of 5.1 million, this is necessary in order to maintain a certain routine. Regardless of the size of the tumour, we commence treatment via the translabyrinthine approach and with increasing experience we are trying in more and more cases to remove even the large tumours by this route. Among the first 13 tumours, four were 3–4 cm in diameter. The remnants were removed by the suboccipital approach, and only one tumour, measuring 2½ cm, was removed by the translabyrinthine route. Among the subsequent 26 tumours we have removed by the translabyrinthine route, *in toto*, six tumours measuring 3½–4 cm. Four measuring 4½–7 cm (Fig. 1) were removed in two stages. Among the 8 tumours removed most recently, three were 4½–5 cm and none needed two stage surgery. Thus, our increasing experience has clearly given more courage, so that gradually we are removing tumours up to 5 cm via the translabyrinthine approach. With respect to preserving and obtaining normal facial nerve function there is a very striking difference between the two operations (Table I). However, it must be admitted that there will still be tumours so large (Fig. 1) that they cannot by any means be removed *in toto* by the translabyrinthine approach which has its spatial limitations.

These results are roughly the same as those published from other clinics (House, 1968, 1978; Clemis, 1971; Glasscock et al., 1978; Morrison, 1978). Among his last 500 tumours operations House (1978) had a mortality rate of 2.6% and among 173 large tumours, 7%. The total removal rate was 93.2%, and total facial palsy occurred in 13.5%. Our results should encourage other otosurgeons to start using translabyrinthine surgery in dealing with acoustic neurinomas. Apart from the fact that the results are better than with the suboccipital approach, translabyrinthine surgery in Denmark has afforded a great stimulus to a more active diagnosis, and a considerably larger number of especially medium sized tumours are being diagnosed today than was the case merely 2 years ago.

ZUSAMMENFASSUNG

Bei translabyrinthären und translabyrinthär suboccipitaler Entfernung der 47 Akustikustumoren war die gesamte Letalität 2.1%, bei 18 großen Tumoren 5.6%. Der Fazialis wurde bei 89% der Patienten geschont und 80% hatten normale Funktion. 98% der Tumoren wurden total entfernt, doch wurde bei 17% der Tumorrest durch den suboccipitalen Zugang eine Woche nach dem translabyrinthären entfernt.

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RECENT EXPERIENCE IN THE SURGERY OF ACOUSTIC NEURINOMAS

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Abstract A translabyrinthine method was used in 49 and a suboccipital approach in 55 cases for the removal of an acoustic neuroma The translabyrinthine procedure is well suited in cases with no pontine compression whereas in large tumours the wide opening in the suboccipital method gives added safety to the procedure The mortality rate was 3% and the facial nerve function after translabyrinthine surgery showed permanent paralysis in 4 patients A team approach using the suboccipital route has been started in an attempt to save hearing in small and medium sized tumours

In recent years otoneurosurgeons have entered the field of acoustic neurinomas, which was previously the preserve of the neurosurgeon (House, 1968) At the University Hospital, Helsinki, the two departments have developed a joint approach, and in this paper we give both a general review of current practice in the handling of acoustic neurinomas and our personal experiences with the various approaches

Geographically, our two departments lie about a mile apart a circumstance which has made it necessary to establish reasonably clear dividing lines Thus small and medium sized tumours are operated on at the Department of Otolaryngology (T P) and the large and very large ones at the Department of Neurosurgery (H T) The preoperative decision has usually been made on the basis of contrast studies, if the 4th ventricle is dislocated the patient is operated on by the neurosurgeon otherwise by the otologist The combined approach is reserved for small tumours in the hope of saving useful hearing, particularly in patients with bilateral tumours

Large acoustic neurinomas show up well in computer tomography, but when they are under 2 cm in diameter, other methods have to be used At the moment it is our firm opinion that positive contrast cisternography, either with iodized oil or with metrizamide, is essential for very small tumours, it seems that metrizamide penetrates better into the meatus In doubtful cases cisternoscopy is a useful procedure after mastoidectomy and exposure of the sigmoid sinus the dura is reflected forward together with the endolymphatic sac and the mouth of the meatus is examined with telescopes The procedure is scarcely more risky than simple mastoidectomy and allows the removal of arachnoid cysts and the cutting of adhesions

For tumour removal without regard to hearing, the methods are well established whether the approach is translabyrinthine or suboccipital The tumour starts growing in the internal acoustic canal, and slowly pushes the arachnoid in front of it, so that all the important vessels lie outside the tumour proper The translabyrinthine approach is ideal for small and medium sized tumours, even some large tumours can be removed *in toto* though there is no doubt that the suboccipital approach allows better exposure for these tumours Should the otoneurosurgeon run into a tumour which cannot be removed *in toto* via a translabyrinthine approach, it is essential that the second operation, through a suboccipital approach, be carried out as soon as possible, before adhesions and scar tissue have

Table I *Results of acoustic neuroma surgery*

Tumour removal	Trans labyrinthine approach	Sub-occipital approach
Planned decompression	5	2
Subtotal removal	8	3
Total removal	36	50*
Total 104	49	55

* 3 after partial translabyrinthine removal 2 were done late and facial nerve could not be preserved One was done at 2 weeks with preservation of facial nerve

formed We think that 2 weeks is the outer limit of the interval between the two operations

The translabyrinthine exposure gives an ideal approach to the internal acoustic meatus and allows precise dissection of the most lateral parts of the tumour, which sometimes present difficulties via the suboccipital approach Morbidity after translabyrinthine removal of the tumour is generally mild and related only to ablation of the vestibular system The facial nerve is identified within its canal and followed towards the pons With small tumours the bleeding is practically nil as all vessels feeding the tumour are first occluded by bipolar cautery and then cut In medium sized tumours, removal of the inner part is recommended before the capsule is freed—and in large tumours such hollowing out is mandatory During the removal the capsule is slowly teased off surrounding structures, at intervals more of the contents are removed to provide as much space as possible for working around the periphery of the tumour Vessels feeding the tumour must be cauterized as soon as they have been identified

The suboccipital approach is routinely used by most neurosurgeons, it is the exposure of choice for large and very large tumours, as it allows an unobstructed view on to the lower posterior pole of the tumour, and the important vessels round this pole and on the surface of the pons can be identified best from this angle and teased off the tumour capsule Because of the larger area exposed, it is easier to control any unexpected bleeding, retracting the cerebellum gently instead of resecting it reduces the danger of cerebellar ataxia However, identification of the middle part of the facial nerve is difficult with large tumours, as frequently the nerve can be seen only when the greater part of the tumour has been removed and, even if there is no pre operative facial palsy, the nerve is often stretched taut and thin and found to be adherent to the capsule The suboccipital approach has recently been well described, so need not be further described here (Yasargil et al , 1977, di Tullio et al , 1978)

The middle fossa approach is usually reserved for intracanalicular tumours or those where the tumour extends no more than 1 cm into the cerebellopontine cistern So far some 20 operations using this approach have been reported, with preservation of hearing (House, 1978)

Recently we have tried a combined approach by otologist and neurosurgeon in the hope of preserving hearing in patients who still have serviceable cochlear function This arose particularly for 2 patients with bilateral tumours, we did not succeed, but think it promising nevertheless The purpose of the combined approach is to allow access to the inner meatus without injuring the labyrinth, here the special experience of the otologist with surgery of the petrous bone is essential A sub-

Table II *Complications of acoustic neuroma surgery*

Complication	Trans labyrinthine approach	Sub-occipital approach
Permanent facial paralysis	4	28*
Temporary facial paralysis	15	22
Intact function	30	5
Cerebellar ataxia	2	4
Early death	2	1•
Cerebrospinal fluid leak requiring revision	1	2

* 5 facial nerves sutured intracranially 3 have shown function
• In addition 5 late deaths

4 783856

occipital craniotomy is carried out as usual, with bone removed to the midline, mannitol and hyperventilation are used to reduce the volume of the brain, and the cisterns are emptied The dura is removed from the posterior surface of the meatus with diathermy, and the bone is drilled off with a Stryker burr, beginning medially at the posterior lip It is important to use the cerebellar retractor so that the otologist looks from as medial a point as possible, since this allows a good drilling angle on to the posterior semicircular canal The tumour mostly originates from the inferior vestibular nerve, so the superior nerve is located first and the separation of the tumour is started from its central margin The first rule is to work gently with a small ear canal elevator and to tease out the tumour without occluding the blood vessels following the cochlear nerve just behind the inferior vestibular nerve The second rule is never to pull the cochlear nerve towards the midline, as its attachments at the Rosenthal's Canal are extremely fragile and the nerve may be torn out even without disturbing the blood supply The preparation must be carried out towards the cochlea as the tumour is freed in the canal The superior vestibular nerve is seldom affected in tumours small enough to allow hope of preserving hearing, but if it is it should also be cut to facilitate removal of the tumour in a lateral direction

Our combined series of acoustic nerve tumours are shown in Table I The number of incomplete removals with the translabyrinthine approach derives from the period 1970–74 when one of us (T P) was working in a hospital without a neurosurgical department The favourable figures for facial nerve preservation (Table II) with the translabyrinthine approach are attributable more to the small size of the tumour than to the approach as such from 1974 onwards the large and very large tumours have been removed via the suboccipital approach, their size makes it natural that this approach carries a greater morbidity, and certainly a greater late mortality Of 2 patients who died after translabyrinthine surgery, one had an intrapontine haemorrhage (a 76-year old man in whom a planned partial removal was carried out), the other was a posterior fossa haematoma (a 54-year-old woman with a large tumour) One patient, who died after suboccipital surgery, had an intrapontine haemorrhage Five patients have died between one month and one year after suboccipital surgery, 2 of persistent infections of the central nervous system, 3 others were so debilitated that they succumbed to extracranial complications

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VESTIBULAR NEURECTOMY

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Abstract Vestibular neurectomy technique used for the standard middle fossa approach and for a total translabyrinthine VIII nerve section is described The middle fossa approach has been used in 49 cases of mainly Meniere s disease and basically the same approach for nerve decompression has been used in 19 cases of facial paralysis 15 being due to Bell s palsy 2 to petrous pyramid cholesteatoma and 2 to facial nerve Schwannoma In unilateral Meniere s disease with no further fluctuation in hearing vestibular neurectomy gives good results in alleviating vertigo

Vestibular neurectomy was employed for the relief of vertigo in a considerable number of patients by Dandy (1941) who used the suboccipital approach to perform the nerve section This was before the advent of antibiotics and microsurgery and although the method worked very well in Dandy's hands (mortality rate 25%), the operation was considered too formidable and fell out of practice It remained for William House (1961) to revive this surgical technique He used the middle fossa approach which in recent years has become one of the standard procedures in hospitals where oto neurosurgery is practised In its present form, section of the superior and inferior vestibular nerves in the internal acoustic meatus is mainly used in the treatment of Meniere s disease and in severe cases of vestibular neuronitis It can also be employed to relieve a persistent positional vertigo which is due to irritation of the posterior semicircular canal For the latter, section of the singular nerve via the middle ear is a logical procedure from a neurological point of view but it so jeopardizes the inner ear function that total vestibular ablation in the meatus is safer In certain ears distressing vertigo following otosclerosis surgery may also warrant vestibular neurectomy This is especially true if hearing has so improved as to reach serviceable levels, no fistula is present, but vertigo remains distressing

Vestibular neurectomy in an extended form is done via the translabyrinthine approach which necessarily sacrifices all remaining hearing This may be advantageous if the ear already has such poor hearing that it merely distorts the speech signals and if, in addition, there is disturbing tinnitus Sectioning of the cochlear nerve is then included in the procedure and only the facial nerve is left in the canal

Technically the middle fossa approach is well standardized Craniotomy in the temporal fossa is done nearly to the level of the floor of the middle fossa and after four burr holes have been made, a 3×5 cm large piece of bone is removed by means of a Stryker craniotome, and a small cutting burr at the inferior edge A 2–3 mm long opening in the dura through the arachnoid is next made to allow some of the spinal fluid escape The temporal lobe dura is gently elevated from the bone as far as the edge of the petrous pyramid to the level of the superior petrosal sinus

It is easy to locate the internal acoustic meatus in non pneumatized petrous pyramids by first locating the superior semicircular canal (Fisch, 1973) and by drilling directly to wards the meatus along a line forming a 60° angle with the canal In pneumatized pyra mids, in which the vertical canal is obscured by air cells, orientation can be safely carried out only by locating the major petrosal nerve first, as originally suggested by House (1961



Fig 1 Various stages of translabyrinthine total neurectomy (A) The vestibular nerves (*arrow*) have been cut The facial nerve is seen at left the cochlear nerve at right (*black arrow*) (B) The cochlear nerve has been cut (*arrow*) and the distal part is ready for extraction (C) Anterior inferior cerebellar artery (*arrow*) high up in the meatus Vertical crest is marked by a white arrow

This nerve is then followed to the geniculate ganglion and along the facial nerve the meatus can always be found Before starting the actual drilling in the meatus, one should make sure that the retractor blade is securely inserted under the pyramid edge and that its angle is at a sufficient distance away from the middle fossa floor to allow enough drilling space and an unobstructed view

The amount of bone drilling varies considerably and at times a very deep hole has to be made before the meatus is reached The important point here is to enlarge the hole along the facial nerve side anterolaterally, taking care not to do too much thinning of the posterior lateral edge because one may inadvertently open the vertical canal Once the dura in the internal acoustic meatus has been encountered, the remaining bone is removed until the transverse crest is clearly visible

The dura is opened along the posterior meatal margin with a sickle knife This exposes the superior vestibular nerve and by turning the dural flap forward the facial nerve is brought into view The vestibulo facial anastomoses are next cut with a sickle knife or with microscissors Particular attention has to be paid to the possible presence of the anterior inferior cerebellar artery (AICA) in the meatus In our series AICA has been found high in the meatus in two cases and but for routine attention to its possible presence it might have been injured

The superior vestibular nerve is then cut at its distal end, reflected laterally and cut again in the area of Scarpa's ganglion The inferior vestibular nerve lies so deep in the meatus that it is best avulsed with a hook and cut centrally with scissors whereupon the rest of Scarpa's ganglion is removed During removal the pieces of nerve are handled as carefully as possible to avoid artefactual changes and are immediately placed in glutaraldehyde solution for later study

Any possible bleeding points are cauterized with bipolar cautery, the canal packed with temporal muscle, the dural fistula closed and the brain allowed to expand Any possible oozing surfaces are covered with Oxygel gelatin cottonoid, and the removed bone put back in its place The drain is always placed directly on bone to preclude any possibilities of extra-dural hemorrhage

The translabyrinthine approach differs from the middle fossa approach only in the manner of locating the nerves in the meatus and in that the cochlear nerve is also removed The mastoid area is obliterated with abdominal fat

Our present series of vestibular neurectomy includes 49 cases and in addition, the middle

fossa approach has been used for total facial nerve decompression in 19 cases Fifteen of the latter were cases of Bell s palsy, while 2 had a petrous pyramid cholesteatoma and 2 a facial nerve Schwannoma Morbidity following neurectomy consists in considerable vertigo for the first 2 postoperative days whereafter compensation occurs gradually The patients are generally fit to return to work after 2 months

Results from surgery for vertigo have been good (Palva et al , 1978) and we employ vestibular neurectomy as a routine procedure for incapacitating vertigo in patients whose hearing in one ear is permanently depressed and non fluctuating For early cases, endolymphatic sac decompression combined with drainage is first tried As regards disappearance of tinnitus, the results are equivocal and cochlear nerve avulsion should be performed only if hearing in the ear has already become useless

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COMPARISON OF THE HEARING THRESHOLD MEASURED BY PURE-TONE AUDIOMETRY AND BY BEKESY SWEEP AUDIOMETRY

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Abstract The hearing thresholds of 115 subjects workers in a shipyard were determined both by Bekesy sweep audiometry and by conventional individual pure tone audiometry at fixed audiometric frequencies The Be

that the standard deviations of hearing thresholds obtained under similar conditions in a pure tone investigation are about twice as large as those obtained in a Békesy investigation

In an impulse-noise project at Kockums Shipyard where we try to measure occupational hearing loss, the principal purpose is to discover significant changes in the hearing [illegible] as a function of noise exposure time (Erlandsson et al, 1978) It is therefore important to know how large the variations in the hearing threshold measurements are

This paper gives the results of the reproducibility and of the relation between hearing thresholds obtained with manual pure-tone and self recording Bekesy sweep audiometry for subjects with varying degrees of hearing impairment

MATERIAL AND METHODS

The hearing threshold of 115 subjects, aged 25 to 63 years, from Kockums Shipyard were determined by both Bekesy sweep audiometry and pure-tone audiometry at fixed audiometric frequencies after 16 h rest from excessive occupational noise A Bekesy audiometer type Demlar 120 and a sound insulated box were installed in a standard caravan The attenuati[illegible] rate was 2 5 dB/s with pulsed tone-presen[illegible]tion and the sweep time from 250 to 10 k[illegible] was 400 s The pure-tone audiometry was p[illegible]formed by the same audiometrist in 5 d[illegible] steps

RESULTS

Comparison between hearing thresholds obtained with pure-tone and Bekesy-sweep audiometry

The hearing thresholds has been determin[illegible] for 230 ears The regression equation for B[illegible]kesy threshold (HL_B) on pure tone thresho[illegible] (HL_T) for the audiometric frequencies 1–6 k[illegible] is $HL_B = -3.4 + 0.93\ HL_T$ and for pure tone [illegible] Békésy is $HL_T = 8.0 + 0.96 \times HL_B$

Test reliability of Békésy-sweep audiograms

In this investigation 10 subjects were mea[illegible]ured five times with an interval of at least 24 The pooled estimate of the variance was co[illegible]puted by first obtaining the variance for ea[illegible] ear and then the mean value for all 20 ea[illegible] was calculated The standard deviations ha[illegible] their lowest values for 1 kHz, increasi[illegible] slowly towards lower and higher frequencies

Reproducibility in Békésy and pure tone audiometry

According to Madansky (1959) it is possible [illegible] estimate the standard deviation of the pur[illegible] tone threshold values if we know the standar[illegible]

deviation of the Békesy threshold values and the regression equation for pure tone on Bekesy threshold at the different audiometric frequencies

Standard deviation (dB)	Frequency (kHz)							
	0 5	1	1 5	2	3	4	6	8
Bekésy	3 2	2 4	2 8	3 5	2 8	3 3	3 4	4 2
Pure tone	6 3	6 2	6 5	4 7	6 6	5 3	7 3	10 4

DISCUSSION

The hearing threshold is not a very well-defined dividing line between hearing and not hearing This uncertainty is illustrated by the swinging in the curve of the Békésy audio meter, where it also is possible to determine the ability of the subject to state the threshold In pure tone audiometry and particularly where the steps are 5 dB and where the threshold is defined as the reading where the subject marks 2 out of 3 bursts, the uncertainty is great The result shows that there is a significant difference between thresholds measured by Bekesy and by pure tone audiometry, which is in good agreement with results obtained in other investigations (Burns & Hinchcliffe, 1957, Harris, 1964 Delany et al , 1966, Knight, 1966)

CONCLUSIONS

The comparison between the two types of audiometry shows that in all cases Bekésy audiometry gives a lower and more reliable hearing threshold

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BSER-AUDIOMETRY IN DIFFICULT-TO-TEST PATIENTS

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Abstract The results of BSER audiometry in a group of 38 difficult to test patients are reported and the response thresholds compared with previous assessments by conventional audiometric techniques The frequently severe behavioural disorders of these patients necessitated the use of general anaesthesia Myringotomy revealed middle ear fluid in 20% of the ears whilst 26% had significant ocular pathology

Electric response audiometry is a technique which permits response assessment at different levels of the auditory pathways Electrocochleography (ECochG) permits recording of electric responses from the organ of Corti and the acoustic nerve, and has previously been widely used (Portmann et al, 1967, Eggermont et al, 1974) The application of ECochG to a group of difficult to test children has recently been reported (Bergholtz et al, 1977) In [illegible] attention is now being paid to the [illegible] events which occur during the first 10 ms after stimulus onset These brain stem electric responses (BSER) can be recorded with surface electrodes, and are generally regarded as originating in the auditory relay centres up to the level of the inferior colliculus (Jewett & Williston, 1971, Lev & Sohmer, 1972, Starr & Hamilton 1976)

Since BSER audiometry has twofold application estimation of response threshold (Sohmer & Feinmesser 1973 Davis & Hirsh, 1976), and topodiagnosis of neurological disease in the posterior fossa (Starr & Achor, 1975, Stockard et al 1977), we have elected to use this technique in the clinic This report presents the results of an investigation of a group of difficult to-test patients with BSER audiometry

MATERIAL AND METHODS

BSER audiometry has been performed in a group of 38 patients, all of whom were either difficult or impossible to assess by conventional audiological techniques All examinations were performed under light general anesthesia, the protocol including routine otomicroscopic examination and, when indicated an ophthalmological assessment

Ordinary ECG skin electrodes were applied at the frontal hairline in the midline, and over each mastoid process The differential bioelectric activity from the ear under investigation was amplified by a DISA 14C13 amplifier with 100 dB gain, filtered with band pass setting of 200–2000 Hz, and led both to a Nicolet 1072 Signal Averager and an HP 3964 FM tape recorder Sweep time was either 20 or 50 ms the inter stimulus interval was 50 ms, and 2048 responses were summated The summed responses were photographed directly from the oscilloscope screen, and later written out on an HP 7045A X–Y recorder

The acoustic stimuli were short, fixed phase tone bursts at octave intervals from 500 to 4000 Hz, generated by a Grason Stadler 1200 system The 4 kHz acoustic stimulus had 1 ms rise and fall times and no plateau, larger stimulus envelopes being used at the lower frequencies The electric signal activated a μ metal shielded TDH 39 telephone in an Amplaid headset Response thresholds were determined visually for both ears

RESULTS AND DISCUSSION

A total of 38 patients has been examined with BSER audiometry in the course of the past

year The mean age was 5 years, and only 6 were over the age of eight Thirteen patients fulfilled commonly accepted "at risk" criteria during pregnancy and the perinatal period (Hirsch & Kankkunen, 1974), whilst a further 8 cases were referred for assessment following postnatal trauma or infection, or the development of manifest neurological deficit Significantly delayed speech development was the principal indication in 17 (45%) of the cases

Many of these patients had profound psycho-motoric disturbances, and were difficult to examine both audiologically and clinically Pre anaesthetic otoscopy was unsuccessful in 28 of the 76 ears Diagnostic myringotomy was performed on 30 ears on the basis of the otomicroscopic findings, middle ear fluid being found in 15, or 20% The secretory otitis media had been clinically unsuspected in 11 of these ears, and altogether 9 grommets were inserted The importance of recognizing concomitant middle-ear pathology in children with sensory neural deafness has previously been mentioned in the literature (Sellars, 1969) Coincidental visual defects are well known to occur both in hereditary and acquired forms of hearing loss (Konigsmark & Gorlin, 1976), and ophthalmological examination revealed significant changes in 10 of the 38 (26%) in this series

Since the auditory brain stem responses are scarcely affected by sedation (Sohmer et al , 1978) or even coma (Starr & Achor, 1975), the use of general anaesthesia should not invalidate threshold estimations The results of earlier audiological assessment have been compared with the BSER thresholds Complete agreement was obtained in the group previously regarded as probably having normal hearing and in the one patient assessed as being deaf BSER thresholds indicated normal hearing in 12 of 22 patients earlier regarded as having some degree of hearing loss, and in 4 of the 7 cases in whom an estimation of auditory capabilities had proved impossible Based on the results of BSER examination, four hearing aids have been prescribed, and two withdrawn Subsequent progress in these patients would seem to confirm the reliability of these decisions

A tentative pure tone audiogram had earlier been obtained in only a very few of these patients, and in these exceptional cases the BSER audiogram has provided confirmation of the behavioural thresholds, especially at the higher frequencies (Clemis & Mitchell, 1977) The disadvantages of this technique are the need for sedation or anaesthesia, and the duration of the examination, which is approximately one hour per patient However, conventional audiometry in this group of patients is also extremely time consuming, and usually must be repeated at frequent intervals The undoubted advantages of precise otomicroscopic and ophthalmological assessment constitute an important, secondary benefit, which revealed pathology in an unexpectedly high percentage of cases in this series

ZUSAMMENFASSUNG

Die Resultate von BSER Audiometrie in einer Gruppe von 38 schwer zu untersuchenden Patienten sind rapportiert und die Befunde der BSER Audiometrie sind mit den Befunden der gewöhnlichen audiometrischen Untersuchungen verglichen Die meisten stark geistig beschädigten Patienten haben Narkose benötigt um die Untersuchungen durchführen zu konnen Paracentese hat in 20% der untersuchten Ohren Flussigkeit gezeigt wahrend 26% der Patienten auch pathologische Augenbefunde aufwiesen

A list of literature references may be obtained from the authors on request

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CHRYOTHERAPY IN THE TREATMENT OF SUBGLOTTIC HEMANGIOMA IN INFANTS

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Abstract Congenital laryngeal hemangioma in infants is situated typically in the subglottic region and causes respiratory obstruction at the age of 1–6 months Hitherto fewer than one hundred cases have been reported The mortality has been high and the therapy problematic As a primary measure tracheotomy must be performed In the treatment of the tumour itself large doses of corticosteroids sclerosing agents radiotherapy and surgical removal have been used The results have hardly been encouraging In five cases we have utilized cryotherapy The hemangioma disappeared after 3–6 sessions No therapeutic or late complications were connected with this mode of therapy

The infantile laryngeal hemangioma is situated typically on the one side of the subglottic area immediately below the vocal cords It appears as a soft, compressible mass, seldom as a solid tumour Histologically the tumour may be capillary or cavernous The colour ies from the pink of normal mucosa, to bluish, depending on the vascularity of the tumour Hemangioma is presumed to develop *from remnants of mesodermal tissue* The exact etiology is not known

The incidence of the infantile subglottic hemangioma is not exactly determined It is considerably rarer than the laryngeal hemangioma in adults which in addition is situated in the glottis or in the supraglottic region In the literature hitherto about 100 cases have been reported In the material of Hollinger & Brown (1967) out of 846 cases of congenital laryngeal anomalies 13 were subglottic hemangiomas

DIAGNOSIS

The diagnosis is based on the clinical history and endoscopic findings Generally there are no symptoms at the time of birth, but the appear at the age of 1–6 months in the for of laryngeal obstruction and are caused by th enlargement of the tumour due to canalizatio of the endothelial tissue The dyspnea is inte mittent and usually worsens during exertio The first symptoms often appear in conne tion with respiratory infections Spontaneo gradual involution of the tumour after 1 months of age is to be expected

In a typical case, no biopsy is needed Su gical intervention can furthermore lead t severe bleeding, as in one of our own case in which it was necessary to leave a tempo in the subglottis In roentgenograms a non specific subglottic mass might be visible

TREATMENT

Tracheotomy must be performed if the tumou narrows the subglottic lumen significantly The treatment of the hemangioma itself has been a problem and no uniform mode of therapy has existed Because of the natural tendency toward regression after one year of age, conservative treatment has been employed Decannulation has been possible in these cases at the age of 14–51 months (Feuerstein, 1973)

Low voltage radiation therapy was earlier generally accepted after Ferguson & Flake (1961) had reported a series of 17 patients with only one death Previously the mortality rate had been 50% The recommended doses were 300–1200 R However, because of possible late complications this mode of therapy has been abandoned In addition, some authors

Table 1 *Cryotherapy in treatment of subglottic hemangioma in children*

Case	Age of diagnosis (months)	Number of applications	Duration of tracheotomy (months)	Follow up time (years)	Comments
1	2	4	4	8	Tracheotomized 18 months before cryotherapy
2	2	6	3	5 1/2	Corticosteroid therapy without effect
3	2	5	2	4	
4	2	6	3	3 1/2	*Residivism (no active treatment needed)*
5	4	3	2	3 1/2	

found no effect on the hemangioma after such small doses In the patients of Ferguson & Flake, duration of tracheotomy was 7–30 months Implantation of radioactive materials directly into the tumour has also been tried (Bruce, 1978)

In a few cases, surgical removal by laryngofissure (Campbell et al , 1958) has also been carried out This can result in scar stenosis, as does the injection of sclerosing agents (Pierce, 1962) Corticosteroid treatment, which is known to accelerate involution of cutaneous hemangiomas, has also been utilized (Overcash & Putney, 1973) The doses must be so large, however, (2–5 mg/kg per 24 hours) that they cause systemic side effects

MATERIAL AND METHOD

During 9 years, from 1960 to 1978, 5 infants with congenital subglottic hemangioma (Table I) were seen and treated at the otolaryngological departments of University of Oulu and University of Kuopio In our material we have applied cryotherapy using nitrous oxide apparatus with a working temperature down to about −40°C or −70°C The aim of freezing was to thrombose the hemangioma and to make it fibrose After the elective tracheotomy the tumour was frozen down to −40°C (the first patients) or to −70°C for 30–60 sec

The applications were carried out through a laryngoscope using a nasal cryoprobe The sessions were repeated at intervals of 2 weeks The hemangiomas disappeared after 3–6 applications Six freezing sessions were needed in a case in which the process was situated at the posterior wall and had to be frozen with caution to avoid the risk of a tracheo-oesophageal fistula (case 4) The duration of the tracheotomy varied from 2 to 4 months (average of 2 8), which is remarkably shorter than by all other modes of therapy The first patient (case 1), however, was tracheotomized and under observation 8 months before cryotherapy was started One tumour (case 4) recurred 2 months after decannulation The patient suffered from respiratory difficulties in connection with infections The attacks of dyspnea were treated with corticosteroids and they disappeared during the following 3



Fig 1 Subglottic hemangioma under the right vocal cord in a 4 month-old boy (case 5)

months Other complications were not recorded None of the patients has shown disturbances of laryngeal development or predisposition to stricture during the follow up periods from 8 to 3½ years

ZUSAMMENFASSUNG

Das kongenitale laryngeale Hemangiom bei Kleinkindern hat seinen typischen Sitz in der Subglottis und verursacht zunehmendes Dyspnoe in dem Alter von 1–6 Monaten Bisher sind weniger als einhundert Falle angemeldet worden Die Mortalitat ist hoch gewesen und die Therapie [illegible] Mittel Radiotherapie und chirurgische Exstirpation verwendet worden Die Resultate sind kaum ermutigend gewesen Wir haben in fünf Fallen Kryochirurgie angewendet Das Hemangiom verschwand nach 3–6 Behandlungen Die Therapie war mit keinen Komplikationen behaftet

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DIFFUSE SCLEROSING OSTEOMYELITIS OF THE MANDIBLE

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Abstract Diffuse sclerosing osteomyelitis of the mandible is a disease which requires a synthesis of clinical radiographic and histologic findings for a correct diagnosis The biopsy technique is important 99mTc scintigraphy and enzyme histochemistry can assist the diagnosis Antibiotics and cortisone are the treatments of choice Decortication should be reserved for recalcitrant cases

Diffuse sclerosing osteomyelitis of the mandible (DSO) is an uncommon disease which always creates diagnostic and therapeutic problems It is described mainly in dental literature (Shafer, Hine & Levy, 1974, Pindborg & Hjørting Hansen, 1974) but the reports are few and opinions differ regarding the nature of the disease Because of the liberal interpretation of the definition many reported cases conform better to other disease entities such as sclerotic cemental masses periostitis ossificans, or Garre s osteomyelitis, than to DSO Equally confusing are the numerous synonyms used to describe the disease, such as non suppurative osteomyelitis osteomyelitis sicca primary chronic osteomyelitis

The purpose of these clinical studies were to analyse the disease in greater detail and to try to improve the diagnostic and therapeutic possibilities

MATERIAL AND METHODS

Twenty patients 14 women and 6 men, with DSO of the mandible were observed for several years The age range of the patients was 14–80 years and the age at onset ranged between 2 and 73 years The clinical appearance and therapeutic possibilities were studied Histologic and histochemical findings were investigated Radiographic features in different stages of the disease were recorded Scintigraphy with ^{99m}Tc diphosphonate was performed and compared with the radiographic appearance

Bacteriologic studies were performed with special attention to anaerobic culture technique

RESULTS

DSO of the mandible was found to appear at any age, in both sexes, but was most frequent in younger to middle aged women The disease was often restricted to one half of the mandible, where it could reach a considerable extension, sometimes beyond the mandibular joint The symptoms were repeated episodes of pain and swelling in the affected part of the mandible and occasional trismus Subfebrile temperature and elevated ESR were noted, but other laboratory data were normal Fistulation was never observed The disease showed a pronounced chronic course with up to life long duration but at times the exacerbations tended to be less frequent and in 2 cases spontaneous regression occurred Antibiotic therapy for short periods of less than a month seemed to have only transient effects Decortication which was performed on 6 patients succeeded in halting the disease process in only one case The other 5 patients were also helped by surgery but had relapses within 6–12 months Cortisone therapy was tried in 6 patients A rapid and complete disappearance of symptoms was noted after an initial dose of 20 mg Prednisolon Successive doses were reduced and treatment was terminated within 10 days Relapses occurred in all patients but as a rule with longer intervals and with less severe symptoms than before

Type I

Sclerotic bone with densely packed, often closed, Haversian systems

Type II

Coarse trabeculae and necrotic foci

Type III

Thin trabeculae with occasional osteoblastemata

Type IV

lation tissue ith giant cells

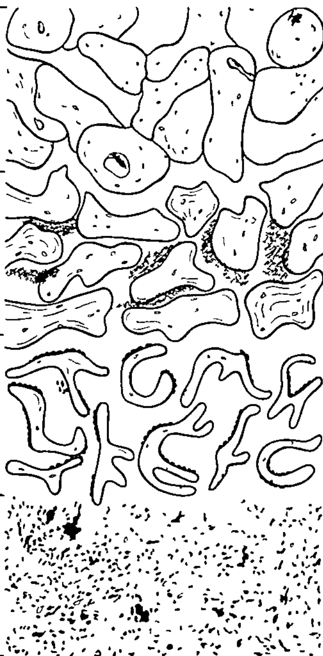


Fig 1 Schema showing the different types of tissue found in cylindrical specimens obtained with a Gidlund's trepan burr

Histological and histochemical investigations, which are discussed in detail in an earlier paper (Jacobsson & Heyden, 1977), showed the necessity of a proper biopsy technique and revealed four different types of bone tissue reaction (Fig 1) The radiographs revealed ill-defined osteolytic zones in a diffuse sclerotic bone Periosteal deposition of bone was an early finding, especially in younger patients Sclerosis was a dominant feature in late stages and in older patients (Jacobsson et al , 1975 and 1978)

^{99m}Tc-scintigraphy showed a high concentration of nuclide in diseased areas, especially in places corresponding to the osteolytic zones found radiographically (Jacobsson et al 1978) Bacteriologic investigations, which are in progress, have not yet been able to prove the role of any microorganism as an etiologic agent

DISCUSSION

DSO is a disease which is mainly confined to be mandible *Other bones in the body may* be involved, but apparently only very rarely (Kopits & Debuskey, 1976) DSO of the mandible has a characteristic but no pathognomonic clinical, radiological and histologic appearance Early discovery is very difficult and the disease is often incorrectly diagnosed as parotitis or pulpitis As pain and swelling occur at more or less regular intervals, however, suspicion should be raised At this stage radiographic changes have usually developed, though sometimes they are very discrete and may be overlooked ^{99m}Tc-scintigraphy then aids diagnosis because of the high uptake of nuclide in diseased parts of the mandible (Jacobsson et al , 1978) The demarcation of the process is also facilitated, which is advantageous when a biopsy or surgical procedure is planned To ascertain the diagnosis and rule out other diseases a biopsy is necessary It is important that the biopsy specimen should comprise both the cortical and the central part of the lesion so that different tissue reactions can be detected A trepan butt is the ideal instrument for this purpose as it makes it possible to take deep and representative biopsies with a minimum of risk of damage of tooth roots, the mandibular nerve, or the facial nerve

The etiology of DSO is obscure but is generally thought to be a low grade bacterial infection (Shafer Hine & Levy, 1974) We have isolated slowly growing propionibacteria from biopsy specimens but contamination has been hard to exclude and serological findings have been contradictory

As long as a bacterial infection cannot be excluded it is advisable to try antibiotic therapy, which should be continued for at least 3 months Cortisone can be recommended to suppress the symptoms at exacerbation Decortication should be reserved for especially recalcitrant cases

ZUSAMMENFASSUNG

Diffus sklerosierende Unterkieferosteomyelitis ist eine Krankheit welche die Synthese klinischer röntgenologischer und histologischer Befunde erfordert um eine korrekte Diagnose erreichen zu können Die Probeexzisionstechnik is wichtig ^{99m}Tc Szintigraphie und Enzymhistochemie können die Diagnose erleichtern Langdauernde Antibiotikatherapie isoliert oder in Kombination mit Corticosteroiden wird in erster Linie empfohlen Dekortikation soll für therapieresistente Fälle reserviert werden

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LATERAL FISTULAE AND CYSTS OF THE NECK

Heredity and Diagnosis

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Abstract A family with congenital fistulae from the second cleft or pouch over four generations is described In order to obtain some idea of how often anomalies are found in relatives of patients with this type of malformation a material embracing 114 patients has been examined The investigation demonstrates that patients with a complete lateral fistula of the neck from the second cleft or pouch showed a heredity of 35% whilst patients with lateral neck cysts did not show any recognizable familial tendency Views on differential diagnosis are presented including the value of fine needle biopsy where in 92% of the cases a diagnosis of branchial cysts of the neck was obtained

In the first month of intra uterine life the developing neck shows a series of branchial arches with grooves in between The internal [illegible]s are called pharyngeal pouches and the [illegible]l ones branchial clefts Eventually the [illegible]s coalesce providing the neck with a [illegible]mooth contour When this amalgamation is disturbed, lateral neck fistulae or cysts occur

A fistula is not always complete It can come to a blind end or develop a cystic expansion either with an orifice on the skin or in the throat so-called external or internal sinuses

A lateral neck cyst may be discovered between the skin and mucous membrane of the throat at any level along the fistula track Usually the neck cyst is found below the sternocleidomastoid muscle s anterior border and externally to the carotid s bifurcation

Histology

A fistula from the second c[illegible]t or pouch in its external portion is outlined [illegible] stratified squamous epithelium but lined with columnar ciliated epithelium in its internal portion Subepithelially, lymphoid tissue is always found and regularly also seromucous salivary gland tissue The fistula track is covered by a thin layer of striped muscle that unites externally with the platysma and internally runs into the palatopharyngeal muscle A cyst from the second cleft or pouch is covered w th stratified squamous epithelium if it lies externally and if it is deeply situated, columnar (sometimes ciliated) epithelium Lymphatic tissue is found around the cyst, whose content is viscous with a high content of cholesterol

Along the whole of its track a cervico auricular fistula is furnished with a stratified horny squamous epithelium In the track, follicles emerge and in the stroma are found sebaceous glands and sweat glands A cyst from the first branchial cleft has the characteristics of a dermoid cyst

A report on kin

The reason for our interest in the hereditary side here was that one of the authors has seven relatives with lateral neck fisulae covering four generations (Fig 1)

The course of the fistula track has in all cases been that typical for neck fistulae from the second branchial cleft and histologically in all cases the picture has been the usual with stratified squamous epithelium externally with columnar epithelium in the internal section of the fistula track and the surrounding lymphoid tissues

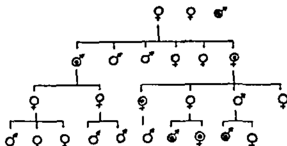


Fig. 1. Kindred. Seven cases of branchial fistulas in four generations of one family.

A perusal of the literature has revealed that a number of authors (among others Bedoni, 1962, Binn, 1965, Bourget, 1966, Rowley, 1969, Wheeler, 1958) have described neck fistulae over several generations but, so far as we have been able to discover, only one kin up to four generations has been reported (Böhme, 1960). Often described in these articles is the simultaneous occurrence of pre-auricular fistulae, deformed outer ears (floppy ears), pre-articular pits, and conductive hearing loss due to malformation of the ossicles, and mandibular hypoplasia suggesting simultaneous involvement of the first two branchial arches.

In the kin described here, in addition to the fistulae from the second branchial cleft or pouch, there are also pre-auricular fistulae in 3 cases and in one case, a slight degree of mandibular hypoplasia. All of them have undergone audiological investigation but no case of conductive hearing loss has been demonstrated and no defect of the ossicles seems likely in the material.

The inheritability of lateral neck fisulae from the second cleft or pouch has been reported (Alasdair, 1973) as being dominant, with reduced penetrance.

Investigation of heredity

In order to gain some idea of how often fistulae or cysts appear in several generations, a retrospective material of 114 patients operated on for fistulae or cysts during the period 1964–77 inclusive at the Regional Hospital, Orebro, has been investigated. Those in the kin reported above are not included in this material, nor are the relatives of the 114 patients operated on. These cases fall into 70 lateral neck cysts, 40 fistulae from the second pouch or cleft and 4 cervico-auricular fistulae from the first pouch or cleft. No case has been traced of fistula or cyst from the third of fourth pouch or cleft (Fig. 2). Of the 70 patients operated on for neck cysts it has been possible to contact 63—it was not considered reasonable to rely entirely on the information given in the case journals with regard to the presence of possible malformations in the kindred. Two of the 63 cases proved to be of interest here, in one of these the maternal grandmother was stated to have had "tuberculosis" on the neck, and in the other case the same was reported for the maternal grandmother's sister. It has been impossible to verify the diagnoses. The remaining 61 people can recall no kin with a fistula or swelling on the neck. Of the 40 patients operated on for neck fistula from the second pouch or cleft it was possible to interview 35. Complete lateral neck fistula was found in 25 of these, whilst the remaining 10 had external sinus. Of these 35, 10 were able to recollect one or more relatives with fistula of the neck. None of the four with cervico-auricular fistula knew of any kindred suffering from fistula or cyst on the neck.

The investigation of the material presented here has thus shown a heredity of 35% for

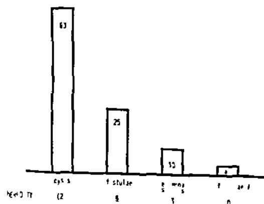


Fig. 2. Material from the Regional Hospital, Örebro, Sweden. Lateral fistulae and cysts of the neck 1964–77.

complete fistulae from the second pouch or cleft but no reliable evidence in regard to lateral neck cysts

Diagnostic value of fine-needle biopsy

Differential diagnosis of fistula with the orifice externally on the neck seldom creates any problems. But when a cystic resistance of the neck is present, there are quite a lot of diagnostic alternatives to consider Although these will not be gone into here it is worth mentioning one patient who was operated for a lateral neck cyst, having earlier been treated with tuberculostatics for a diagnosis of "cervical tuberculoma"

In recent years, in the investigation of suspected cysts, fine-needle biopsy has been used regularly In order to evaluate the diagnostic accuracy the cyst material has been searched for this purpose also Of the total of 70 patients, 55 underwent preoperative-fine-needle biopsy Cytologic investigation produced a preoperative diagnosis of lateral neck cyst in 51 (92%) of these In the remaining 4 patients the cytological diagnoses recorded a reactive proliferative process, malignity, atheroma, and necrotic material These diagnoses have been rechecked and confirmed In the first two diagnoses, inflamed neck cysts were concerned, where the diagnosis cannot always be made without a risk of overdiagnosis The possibility of a cystic transformed metastasis of stratified epithelial cancer must, however, be taken into account Thus in the assessment of the cytology report it is necessary that the cytological diagnosis shall agree with the clinical picture The cytological diagnosis in fine-needle biopsy is made a great deal easier if, when the biopsy is carried out, both air-dried and spirit-fixed material is sent for assessment, accompanied by the clinical picture and the exact location

ZUSAMMENFASSUNG

Nach einer topographischen und embryologischen Vorstellung von kongenitalen Halsfisteln und Zysten wird eine Familie mit kongenitalen Halsfisteln des 2 Kiemengangs in vier Generationen prasentiert Um festzustellen wie oft disese Anomalie bei Anverwandten der Patienten mit dieser Art von Mißbildungen vorkommt wurde ein retrospektives Studium gemacht Die Untersuchung ergab eine positive Heriditat in 35% bei Patienten mit kompletten kongenitalen Halsfisteln des 2 Kiemengangs dagegen keine familiare Haufung bei Patienten mit lateralen Halszysten Differentialdiagnostische Gesichtspunkte werden vorgelegt unter anderem der diagnostische Wert der Franzénpunktion bei lateralen Halszystem wobei die Diagnose in 92% der Falle praoperatif korrekt gestellt war

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ABSCESS TONSILLECTOMY À TIÈDE

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Abstract During recent years primary tonsillectomy tonsillectomy *a chaud* has again become popular as the standard therapy for the peritonsillar abscess whereas the traditional tonsillectomy *a froid* made 4–6 weeks after the incision has been partially eclipsed It seems however, that the intermediate form between these two tonsillec tomy *a tiede* an abscess tonsillectomy made 3–4 days after the incision would be highly practical in many cases compared with the other two mentioned above The ton sillectomy *a chaud* requires such a high state of readiness for anaesthesia even during the emergency hours that it is not practical in all otolaryngological departments Ton sillectomy *à tiede* needs a longer hospitalization yet re quires fewer days off work than does the classical ton sillectomy *a froid* method Furthermore a significant proportion of the patients fail to present themselves for tonsillectomy at the agreed time and consequently get a recurrence of the disease later on Tonsillectomy *a tiede* is almost as easy an operation as the normal tonsillec tomy *both for the surgeon as for the patient*

During the years 1976–77 153 cases of peritonsillar ab scess were seen 105 cases were treated with the tonsill ectomy *a tiede* method The average duration of treatment was 6½ days The only complications were 6 cases of light secondary bleeding A tonsillectomy *a chaud* was per formed on 9 children in the age group 3–9 years

Although the peritonsillar abscess has been effectively treated for more than a century, it seems that there is still no unanimity about the mode of treatment Before the era of antibiotics, tonsillectomy under local anaesthesia in the 'hot phase, the tonsillectomy *a chaud*, gained popularity particularly in Europe (Virtanen, 1949 Grahne, 1958) only to give way after the advent of antibiotics In the "cold" phase, 4–6 weeks after the incision of the abscess made tonsillectomy *a froid* is technically more cumbersome than is the case with a fresh abscess it requires two periods of sick leave and many patients neglect to come for an elective tonsillectomy (Jaakkola & Johanson, 1964, Štermensky & Papsö, 1974) Under these circumstances the frequency of recurrent abscess becomes high Nowadays primary tonsillectomy under general anesthesia has again become popular (Bateman & Kodicek, 1959, Bonding, 1973, Jokinen, Karjä & Nuutinen, 1975 etc) Less attention has been paid to the intermediate form of the hot and cold phase operations—the abscess tonsillectomy *a tiede*, where the tonsillectomy is made in the tepid phase 3–5 days after the incision (Canuyt, 1933)

Tonsillectomy *a tiede* was introduced into the Central Hospital of Kuopio over 15 years ago, because although we realized the advantages of the *a chaud* method, we regarded the local anaesthesia as too troublesome and the general anaesthesia resources of the hospital limited *a chaud* operations to exceptional cases only The purpose of this paper is to illustrate our method and to present its indications in the light of the material of the last 2 years

MATERIAL

During 1976–77, 153 patients were treated because of peritonsillar abscess They also constituted at the same time the bulk of these

Table 1 *Treatment of 153 patients with peritonsillar abscess*

Method	No of cases
Tonsillectomy *a tiede*	105
Tonsillectomy *a chaud*	9
Tonsillectomy *a froid*	21
Incision only	18
Total	153

cases in our central hospital district of 250000 inhabitants. 63 of the patients were female and 90 male. The age incidence ranged between 3 and 74 years, 105 cases (69%) being young adults of 15–30 years. Those 14 years old or younger were 15 (10%) and those 46 years or older were approximately the same (14 cases).

The *method of treatment* is shown in Table I. Tonsillectomy *à tiède* has been our routine therapy. It was also applied to teenagers, for whom both the incision and the tonsillectomy could be performed under local anaesthesia.

Tonsillectomy *à chaud* was regularly performed in those children in whom the incision alone would have made general anesthesia necessary. The average age of these children was 6 years 8 months. No tonsillectomy *à chaud* operation was made on an adult patient during the observation period. The tonsillectomy *à froid* was used for exceptional reasons only, e.g. on the request of the patient or for the closing of the ward due to annual repairs.

We were satisfied with a mere incision in those cases where the patient's general condition or other disease was a contra-indication to the tonsillectomy. This group included also the 4 patients who refused tonsillectomy and hose 6 patients who did not attend for an lective tonsillectomy *à froid* operation.

Tonsillectomy *à tiède* was thus performed on 105 patients, of whom at least 51 (or every second patient) was suffering from recurrent tonsillitis In 10 cases it was a question of a recurrent abscess. In all patients in this group the incision was made at the hospital and parenteral penicillin therapy was commenced. The incision needed manual drainage daily and the tonsillectomy was made when the inflammation had subsided after 3–4 days. With the usual infiltration anaesthesia, using 0.5% lidocain, tonsillectomy was performed satisfactorily in all cases. The average duration of treatment for these patients was 6½ days. The hospital stay was partly prolonged by the weekends falling between the incision and the tonsillectomy. Likewise in some cases a prolongation of the treatment was due to a too cautious incision made by an inexperienced resident. The bleeding frequency was the same as in routine tonsillectomies. A secondary bleeding occurred in 6 cases, 5 of them from the side of the abscess, one from the "healthy" side. The bleedings were easily stopped by means of electrocoagulation. More than half of the patients arrived for follow-up examination 6 weeks after the operation, when every one of them was found to be fully recovered. No serious complications were connected with the treatment in any group.

DISCUSSION

If only the efficiency of the treatment is considered, tonsillectomy *à chaud* must be given preference. However, it does require round-the-clock anesthesia cover. Thus this method is expensive for the hospital. Also the anaesthetic risk is without doubt greater with the starving, thirsty and febrile patient in the *à chaud* phase.

Tonsillectomy *à froid* does not include any remarkable risks and can be performed even in a less comprehensively equipped hospital, but still we do not consider it recommendable. The treatment requires two visists to the hospital and above all two separate sick leave periods. In this way the total period of illness is longer than with the other treatment forms and consequently occasions higher social expenses. It must also be kept in mind that by the *à froid* method a proportion of the patients remain unoperated and the number of recurrent abscesses and tonsillitis cases increase the morbidity. This treatment method is thus for the hospital the cheapest, but for society as a whole, the most expensive. Also it must be remembered that the removal of the tonsils is always more troublesome after the abscess cavity has scarred, than is operation at an earlier stage.

The tonsillectomy *à tiède* is a form intermediate to the other two treatments. It does not have the disadvantages of either of them

The operation is easy and painless, under local anaesthesia, 3–4 days after the incision, irrespective of whether the abscess cavity has completely disappeared or not

The hospital treatment duration in *a tiede* cases is 3–4 days longer than in *a chaud* cases (on average 6½ days), whereas the treatment time in ordinary tonsillectomies as well as in *a chaud* cases is only 3 days, including the days of reception and discharge This advantage disappears, however, if the incision phase is treated at the outpatient department The patient needs also only one sick leave, so the overall duration of sickness does not deviate remarkably from that of the *a chaud* cases Taking into account not only the medical but also the public health and social security aspects, tonsillectomy *a tiede* is a recommendable treatment method for the peritonsillar abscess in most hospitals Signs that this opinion is about to gain in popularity can also be seen in the literature (Stermensky et al)

ZUSAMMENFASSUNG

In den letzten Jahren hat die primare Tonsillektomie die Tonsillektomie à chaud wieder Fortschritte gemacht als die Standardtherapie bei Peritonsillarabszeß und die traditionelle Tonsillektomie a froid 4–6 Wochen nach der Inzision ist teilweise in den Hintergrund gekommen Es scheint jedoch obwohl die Zwischenform für diese Behandlungsmethoden die Tonsillektomie a tiède die Abszeßtonsillektomie 3–4 Tage nach der Inzision in manchen Fällen viel praktischer ware als die beiden obenerwähnten Methoden Die Tonsillektomie à chaud fordert eine so effektive Anästhesiebereitschaft sogar während der Notdienstzeit was nicht auf allen Ohrenabteilungen möglich ist Die Tonsillektomie à tiède erfordert mehr Krankenhaustage aber verursacht weniger Krankentage als die klassische Tonsillektomie a froid Methode Außer dem kommt eine bedeutende Anzahl von Patienten nicht zu der verabredeten Tonsillektomie und erkranken später aufs neue Die Tonsillektomie à tiède ist beinahe ebenso leicht als Operation wie die normale Tonsillektomie so wohl fur den Chirurgen als fur den Patienten

An der Ohrenklinik der Universitat von Kuopio haben wir in den Jahren 1976–77 insgesamt 153 Falle von Peritonsillarabszeß gehabt von denen wir 105 Falle mit Tonsillektomie à tiède behandelt haben Die durchschnittliche Behandlungszeit war 6 5 Tage Die einzige Komplikation waren nur 6 leichtbehandelte Sekundarblutungen Tonsillektomie à chaud wurde nur an 9 Kindern in der Altersgruppe 3–9 Jahre durchgeführt

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RESULTS OF THE TREATMENT OF ACHALASIA BY PNEUMATIC DILATATION

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Abstract Thirty three patients treated at the Karolinska [illegible]

[illegible] sphincteric pressure was calculated before and after dilatation It was found to be higher than normal preoperatively, but reduced to a near normal value postoperatively

Achalasia is marked by the absence of propulsive motility in the esophagus and by incomplete relaxation of the lower sphincteric area

Its symptoms are dysphagia, regurgitation and sometimes retrosternal cramps and loss of weight

The alternative treatments are a myotomy of the circular muscle wall in the lower part of the esophagus through a transthoracal or abdominal approach or an instrumental dilatation of the lower sphincter (LES) with different types of dilators The choice of treatment seems mainly to be influenced by local therapeutical traditions Since the end of the 1930s achalasia patients at the Karolinska Hospital in Stockholm have mostly been treated by pneumatic dilatation (Gjertz, 1963) However, at other therapeutical centres, surgical therapy is recommended and applied (Ellis et al, 1967)

In order to survey the results achieved by pneumatic dilatation and the frequency of complications with this method of treatment, achalasia patients treated at the Karolinska Hospital between 1968 and 1978 have been re examined The effect of dilatation on the lower sphincteric characteristics before and after dilatation has also been studied

METHOD AND MATERIAL

The dilator consists of a mercury-filled bougie with an inflatable hour glass-shaped radiopaque bag (Norris modified Brown McHardy esophagospasm dilator, Pilling Co, USA)

Under local anesthesia the dilator bag is placed in the sphincteric area Its position is controlled fluoroscopically and the bag is inflated to its maximum extent (4 cm) Postoperatively a constrast X-ray is taken in order to ascertain if the esophagus has been perforated

The number of achalasia patients treated during this period was 33 (23 men and 10 women) Age distribution at the time of the onset of symptoms can be seen in Fig 1 Roughly half the patients began to receive treatment within 3 years of the onset of the symptoms

In 6 cases the resting pressure of the lower sphincter was measured before and after the dilatation by a method described by Waldeck et al (1973) The patient swallowed a four hole catheter, which was connected to a transducer and constantly withdrawn at a speed of 5 mm/sec while being perfused at a rate of 5 ml/min By this method a graphical profile of the sphincter is obtained on a mingograph and the maximum resting pressure and length of the sphincter can easily be calculated The procedure is repeated ten times and the average result is calculated for the two parameters

Of the 31 patients who were preoperatively

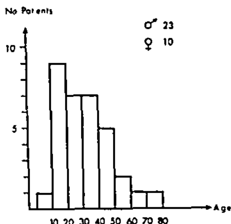


Fig 1 Age distribution at onset of symptoms

examined by X-ray, 24 patients had signs that are typical of achalasia

Twenty-eight patients had been treated by pneumatic dilatation The results were unsatisfactory for 3 of these patients and they were later treated with myotomy

One patient was subjected to myotomy without dilatation having been previously attempted Two patients were treated only with mercury bougies, while the remaining 2 patients were first treated by bougienage and later operated on by myotomy Of the 28 patients subjected to pneumatic dilatation, 20 were treated once, 4 twice and 4 three times There was thus a total of 40 pneumatic dilatations

RESULTS

In the majority of cases the late results after pneumatic dilatation have been judged on the basis of information provided by the patient in person by telephone or by letter The patients were asked about difficulties in swallowing, the frequency of regurgitation and cramps and loss or increase of weight The patients were placed in three groups on the basis of the information they have given

Group 1 patients with excellent results, by which is meant the complete absence of symptoms or only occasional difficulty in swallowing or isolated bouts of retrosternal cramp

Group 2 (good results) comprises patients who regularly experience problems of this kind—though not more than once a week—and who think the treatment has clearly improved their condition

Group 3 (poor results) comprises patients who have experienced only a slight improvement or none at all after treatment

The average follow up period for the first two groups is 35 months (the shortest period is 4 months, the longest 7½ years) The length of the follow-up period for group 3 has not been calculated since the unsatisfactory result was usually apparent immediately after dilatation and a different treatment was adopted

The result was considered excellent in 15 (54%) cases, good in 7 (25%) cases and poor in 6 (21%) cases The groups' excellent and good results therefore constitute nearly 80% of the total

Of the 6 patients in group 3, 4 were dilated once and 2 were dilated three times Another 2 patients whose esophagus was perforated in relation to treatment, have also been included in group 3 Postoperative X-ray examination revealed no sign of perforation in either of these cases Symptoms of perforation appeared during meals taken shortly after the dilatation

Accordingly after this complication postoperative treatment has involved parenteral

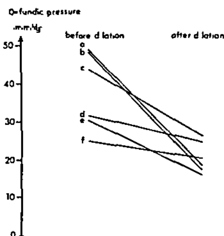


Fig 2 Mean resting pressures before and after pneumatic dilatation

fluid therapy for at least 12 hours after the dilatation The 2 patients were thoracotomized and survived and have at present no difficulties in swallowing The perforation rate thus constitutes 5% of the total number of dilatations Three patients have been subjected to myotomy after unsuccessful dilatation Two of them have since been completely free of symptoms The only patient who underwent myotomy without previous dilatation has also been cured

Fig 2 illustrates the average resting pressure in the lower sphincteric area of the 6 patients in whose cases this pressure was studied before and at least 6 months after dilatation Their mean resting pressure constituted 39 mmHg with a distribution of 26–49 mmHg Using the same technique of measurement, the normal pressure within a group of healthy persons was 19±1 6 mmHg (Waldech et al 1973) The preoperative sphincteric pressure of these 6 achalasia patients was thus above normal After the dilatation the pressure declined in all cases to an average of 22 mmHg with a distribution of 18–28 mmHg Thus the mean pressure is very close to that of a normal person Of these 6 patients 5 were cured of symptoms while one did not improve and later r a myotomy This patient is case (*d*) who had a fairly high sphincteric pressure which declined somewhat after dilatation but none the less remained relatively high

The length of the high pressure zone has been calculated before and after dilatation The average preoperative length of this zone of 34 mm does not differ from the norm which is claimed to be 28–35 mm (Waldech et al 1973) However the postoperative value fell to 25 mm despite a slight elongation in 2 cases

Our experience is that achalasia is an unusual disease which seems to occur at all ages Esophagus manometry is of great value for diagnosing achalasia at an early stage It has been shown in this article that the lower esophageal sphincteric pressure is higher in achalasia sufferers and t s observation is confirmed by other report though utilizing different methods of measurement (Heitmann Wienbeck 1972)

Pneumatic dilatation leads to a good re in almost 80% of achalasia cases Se other reports concerning the same metho treatment show similar results (Kurlande al 1963 Vantrappen et al , 1971)

Similar results are also claimed for surg myotomy (Ellis et al 1967) The common of these two methods is to reduce sphinct pressure A well known complication a myotomy is however, postoperative e phagitis due to reflux and strictural devel ment which is said to occur in 5% to 2 of all operated cases This complication dom occurs after pneumatic dilatation

There are hardly any contra indication pneumatic dilatation This treatment is eas perform and can be repeated if the initial sult is unsatisfactory

Should the need arise, repeated dilata often are performed within a short period time since if the effects of this treatment poor this is usually immediately apparent after two to three dilatations the patient s c dition has not improved a myotomy ough be performed This operation should also considered as primary treatment for youn patients or for patients who cannot be p suaded to cooperate

Moreover in a few cases in which the e phagus has a sigmoid like form and the dila cannot be made to pass the cardia onl myotomy is possible

The frequency of perforation shown in t article may seem high but is confirmed other studies e g Gjertz (1963) The rates complications and recurrence are howe no higher for pneumatic dilatation than th reported for surgical myotomy and can p haps be further reduced in the future

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STRUCTURAL CHANGES IN THE COCHLEA FOLLOWING OVERSTIMULATION BY NOISE

H Engstrom and B Engstrom

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It has long been known that noise exposure of both brief and prolonged duration can cause hearing loss in man It is also known that there is a direct relation between hearing loss and certain industrial occupations It was therefore natural that scientists long ago started animal experiments to elucidate how noise exposure caused hearing loss As early as 1948 Ruedi & Furrer made an extensive study which clarified several important facts related to noise induced cochlear damage Many clinical and experimental studies have followed and the ...tion of modern methods for a structural ... s including surface specimen studies, ...sion and scanning electron micro... well as enzymatic and other quanti... ...dies, has provided us with important ...tion (Stockwell et al , 1969, Eng... l , 1970, 1976, Spoendlin, 1971, ...l , 1972, Bredberg & Hunter ... Bohne, 1976, Soudjin, 1976, ... 1978) Many interesting prob... ...ever, and extensive studies ... in many different labora...

... act upon our ears in dif... ...all familiar with the fact ...noise of even very short ... ear damage This is ...ulse sound, or explo... ...odification caused by ...ll known and can ...n by scanning ... Much less is ...tions inside ... a temporary ... necessary to develop new methods to make a structural or biochemical analysis The temporary threshold shift should in its "pure" form indicate a return of hearing to the original level after exposure However, several authors have described how very difficult it is to prove by pure tone tests in animals that there is not a certain degree of damage Bohne (1976) has taken up what is called asymptotic threshold shift (Carder & Miller, 1971) for discussion and posed the question ' are any long, continuous exposures to noise safe?" In her experiments she used chincillas and could show the effect of short- versus long term exposures (2 and 9 days) She could also show how dramatically an 8 dB increase (from 72 dB to 80 dB) could increase the damage She also found that the pillar cells in the first cochlear turn are nearly as susceptible to noise exposures as are outer hair cells and believed this to be related to the cochlear damage in industrial noise Her conclusion is that the return to normal of a pure tone threshold after exposure to noise does not necessarily indicate that the exposure is innocuous This is a very important statement as it has a direct bearing on the effect of repeated exposures

If our knowledge about minor modifications is unsatisfactory, our knowledge concerning more pronounced hearing loss and its morphological background is rapidly improving especially as the SEM and TEM techniques can be combined (Malik & Wilson, 1973, Hunter-Duvar, 1978)

There is general agreement that sound pres-

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SYMPOSIUM ON NOISE INDUCED HEARING LOSS

Moderator Gunnar Lıden, Sweden

INTRODUCTION

A good workıng envıronment has become ın creasıngly ımportant over the last few years Noıse at the place of work ıs one of the most common causes of dıssatısfactıon regardıng the workıng envıronment In spıte of hearıng conservatıon programs, noıse stıll gıves rıse to the development or progressıon of hearıng loss Thus we may questıon the effıcıency of our present ınjury rısk crıterıa A good workıng envıronment may be saıd to be one ın which the whole populatıon may be exposed throughout ıts workıng lıfetıme wıthout acquırıng an ınduced threshold shıft greater than 5 dB at 4 000 Hz Thıs goal may be reached ıf the 40 year (8 hr/day) noıse exposure level does not exceed 75 dB(A) (von Gıerke & Johnson, 1976) From an economıc vıewpoınt, many ındustrıes may fınd ıt dıffıcult to reduce e noıse to thıs low level However, our task ust be to emphasıze where the safe noıse level lıes It ıs then up to the occupatıonal safety and health admınıstratıon as well as to the polıtıcıans to decıde what percentage of the populatıon we can afford to protect against noıse ınduced hearıng losses

The present panel wıll dıscuss the evaluatıon of the ınjurıousness of ıntermıttent and ımpulse noıse, dosımetry and the equal energy prıncıple The panel wıll also cover morphologıcal, physıologıcal, psychologıcal and clınıcal vıewpoınts on noıse ınduced hearıng loss

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Noise or sound can act upon our ears in different ways We are all familiar with the fact that high intensity noise of even very short duration may cause inner ear damage This is especially true of impulse sound, or explosions The structural modification caused by such noise is today quite well known and can be studied with great precision by scanning electron microscopy (SEM) Much less is known however about modifications inside the organ of Corti in the case of a temporary threshold shift For such cases it is necessary to develop new methods to make a structural or biochemical analysis The temporary threshold shift should in its "pure" form indicate a return of hearing to the original level after exposure However, several authors have described how very difficult it is to prove by pure tone tests in animals that there is not a certain degree of damage Bohne (1976) has taken up what is called asymptotic threshold shift (Carder & Miller, 1971) for discussion and posed the question "are any long, continuous exposures to noise safe?" In her experiments she used chinchillas and could show the effect of short- versus long term exposures (2 and 9 days) She could also show how dramatically an 8 dB increase (from 72 dB to 80 dB) could increase the damage She also found that the pillar cells in the first cochlear turn are nearly as susceptible to noise exposures as are outer hair cells and believed this to be related to the cochlear damage in industrial noise Her conclusion is that the return to normal of a pure tone threshold after exposure to noise does not necessarily indicate that the exposure is innocuous This is a very important statement as it has a direct bearing on the effect of repeated exposures

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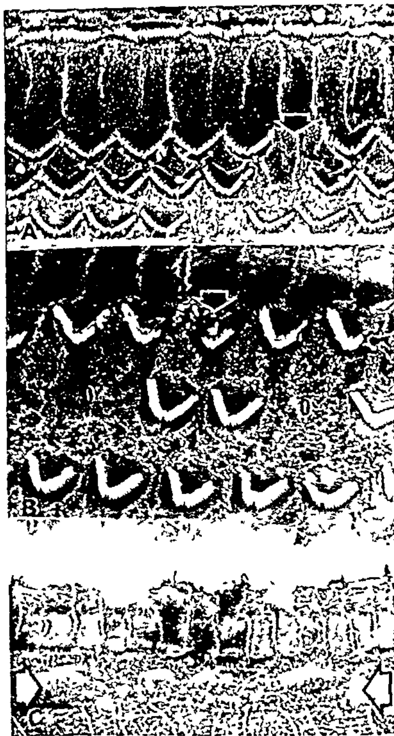


Fig 1 Three SEM micrographs of the organ of Corti in different stages of degeneration after noise exposure In (A) only three cells are lost the remaining cells seem to be in good condition In (B) the damage has caused degeneration of several cells (O) but the hairs on several cells in the first row (*arrow*) are changed by noise In (C) the whole region between the two arrows is destroyed with all the outer hair cells gone while the inner hair cells still remain in fairly good condition

sure level, frequency and exposure duration are the main causative factors in noise damage Rüedi & Furrer long ago came to the conclusion that a pure tone is more traumatic than a noise of the same intensity and duration They further found that the site and extent of a given acoustic trauma show marked individual variations In recent publications too the latter statement has been repeatedly confirmed (e g Dolan et al , 1975) Nevertheless there is a general tendency for the noise damage to follow a frequency related

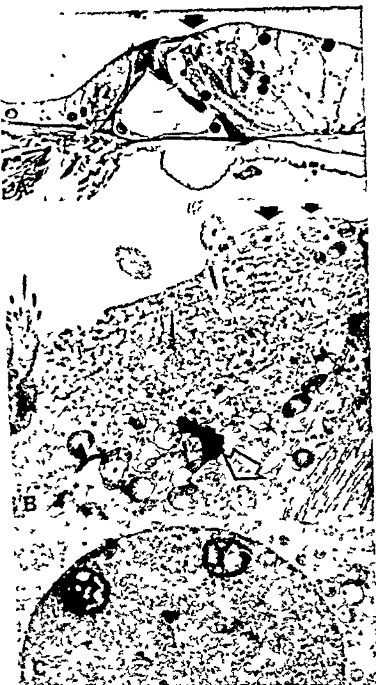


Fig. 2. (A) is a phase contrast microscopic picture of the organ of Corti damaged by noise. All the three rows of outer hair cells (*below the arrow*) are gone. In (B) it can be seen that an inner hair cell has been damaged by noise exposure. The hairs at the surface have become ingested by the cell (*arrow*) presumably to reduce the free surface. Inside the cell degenerated or degenerating (arrow) m...

pattern. Animals exposed to 4 kHz suffer the main damage in the basal coil while 2 kHz damage is found in the middle coil of the guinea pig and 1 kHz still higher up. The extent of the damage is directly related to the intensity.

What happens then to the inner ear in noise damage? It is very difficult to state where the earliest damage occurs but it is quite evident that rather early the sensory hairs on the outer hair cells lose their stiffness, they have a tendency to "wilt" and it is still unre

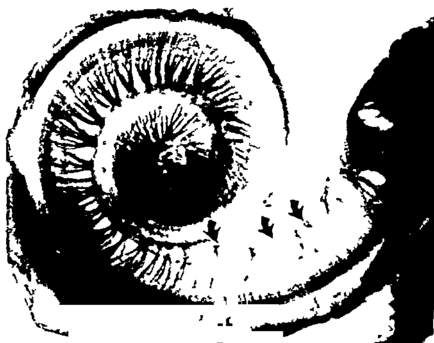


Fig 3 Human cochlea man 64 years At the basal end of the cochlea a distinct reduction of nerve fibres (*arrow*) at least partially induced by noise exposure

solved whether this is a reversible process or not If the damage is sufficient, the hairs may fuse and form conglomerations Some parts of the hairs become ingested into the surface of the outer hair cells and can be found lying horizontally inside the plasma membrane above the cuticular plate In inner hair cells a number of fused hairs can form a spatular plate protruding from the surface Parallel with the modification of the surface various grades of deterioration appear within the hair cell cytoplasm In the case of minor damage the structural changes can result in the formation of lysosomes appearing especially in the region below the basal body It seems probable from our studies that an increase in lipofuscin granules may occur after repeated noise exposures

If the damage is lethal to the cell both nucleus and cell cytoplasm begin to disintegrate Vacuoles or cystic degenerations appear and the cells lose contact with the reticular membrane The gaps are rapidly closed by outgrowth of supporting cells mainly from the Deiters' cells but even the pillar cells participate In the case of less serious injury the outer hair cells are often damaged first (Fig 1 A) and the regular pattern is broken Scattered cells or groups of sensory cells disappear and in more pronounced damage all outer hair cells may degenerate in a more or less restricted area Depending upon the type of noise exposure, the damage can appear as small spotted regions of hair cell loss or as a distinctly localized damage The inner hair cells are often more resistant than the outer ones but in severe exposures large numbers of inner hair cells may also disappear This is often followed by neural degeneration in a corresponding area

In sections through the organ of Corti the fluid spaces are at first preserved With increasing disintegration macrophages appear and the fluid spaces fill with outgrowth from the supporting cells These also disappear as do—after varying periods of time—nerve endings and nerve fibres and even the ganglion cells in the spiral ganglion The rest form a cuboidal layer on the basilar membrane During this process the tectorial membrane is lifted off from the organ of Corti in its damaged area It looks as if the tunnel of Corti can remain intact in undamaged regions but disappears in severely damaged regions

Duvall et al (1974) and several others have made careful studies of the stria vascularis in noise damage and are of the opinion that there is no doubt that temporary but significant structural changes occur in the stria and permanent changes in the spiral prominence The role of the stria damage in relation to the simultaneous organ of Corti damage is of great interest Duvall et al mean that the pathology seen in acoustic trauma in the stria is remarkably similar to that observed following large intravenous doses of ethacrynic acid

The modifications in the cochlea described here do not occur immediately after exposure but need varying time to develop partly depending upon the extent of the damage The fully developed damage may sometimes be seen first after weeks or even months This is especially true of the degeneration of nerve fibres One question of interest that has recently been raised is whether young babies are more susceptible to noise than adults This could be of special interest for babies being kept in the noise produced by incubators where the infant must remain for a considerable time under continuous exposure

A few words should be said about the effects of explosions Depending upon the type of exposure, many different kinds of damage can occur This includes not only cochlear hair cell loss but also drum membrane ruptures ossicular dislocations and bleedings ~~Impulse sound can appear in many forms and~~ impulse noise is of special interest as a causative factor in cochlear damage

When reporting on the damaging effect of sound or noise it is of the utmost importance that appropriate descriptions of exposure of sound sources and methodologies are given This is especially true of environmental noise Several of these requirements have been collected in Guidelines for preparing environmental impact statements on noise (CHABA 1977)

When describing animal experiment results some further factors are of special interest There is much evidence that genetic factors may be of very great importance for cochlear degeneration even without noise exposure In the same way the animal s age may be an important factor in long term experiments

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PHYSIOLOGICAL ASPECTS OF THE EFFECTS OF SOUND ON MAN AND ANIMALS

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Abstract A review of some short term and long term physiological effects of sound on non auditory body functions is given It is pointed out that the short term effects depend closely on the acoustic properties of the sound Habituation is rapid for steady signals but slow for interrupted ones Irrelevant meaningless sound presented to rats over their life time in such a way as to simulate an industrial acoustic environment did not affect blood pressure life span or morbidity incidence As far as the injurious effect of sound on the inner ear is concerned it was shown that spontaneously hypertensive rats were considerably more susceptible to such impairment than were normotensive ones Hence sound does not produce hypertension but susceptibility to ear injury may be greater in hypertensive individuals The potential role which individual variability in physiological properties of the sound conduction system might play for the observed individual variability in noise induced hearing loss is also discussed

In contrast to the effect of sound on the inner ear, non auditory reactions are expected to depend to a large extent on the meaning of the acoustic signal For instance, a meaningful sound such as a warning signal even when weak instructs the body to react adequately to a certain danger An irrelevant or meaningless sound on the other hand, may be defined as sound which does not contain information calling for attention or action One question of basic importance with regard to the non auditory effect of sound is therefore Does sound influence the body even when it does not convey information about anying other than its own presence? If the answer to this question is "yes —if reactions to meaningless sound do exist and persist for a long time—sound must be controlled with respect to its non auditory effect in a way similar to that regarding its adverse effects on the inner ear If the answer is "no"—if long term physiological and pathophysiological alterations are produced only when the sound is perceived as a warning *signal concerning some impending problem* it is not necessary to control the sound on the basis of its physical characteristics Instead attention must then be given to the sources of the warning signals Masking of verbal communication and interference with mental tasks are also important, but will not be considered here

The physiological aspects of the effects of noise are not restricted to non auditory reactions They also concern various physiological conditions which may influence the susceptibility of the inner ear to injury by sound In the following, a survey is given of some studies on short term and long term non auditory effects of sound as well as on physiological factors affecting the susceptibility of the inner ear to noise induced hearing loss The results are based mainly on experiments about which reports have already been published or are in preparation

A *Non auditory effects of sound*

Today there is a general awareness of the harmful effects of sound on the ear Consequently, no high level sound is meaningless strictly speaking since it acts as a warning about possible injury to the ear An analysis of the effects of sound itself thus cannot be made in man—at least not as far as high level sound is concerned Animal experimentation is needed in order to study the effects of sound itself In long term experiments on animals

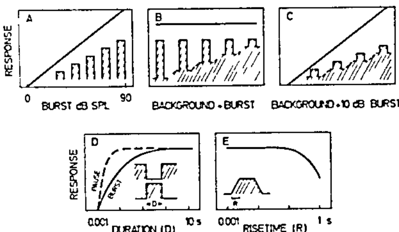


Fig. 1. Simplified outline of the dependence of peripheral vasoconstriction (Response on ordinate) on the intensity of a noise burst (A) on the magnitude of the change of sound level (B, C) on the duration of a noise burst or a pause (D) and on the rise time of a 4 s noise burst (E).

the environmental conditions can be adequately controlled and specified. Several years' experiments on rats have been performed mainly concerning two questions.

1) What properties of the stimulus determine the acute physiological reactions to sound and under what conditions do these reactions occur?

2) What are the long term effects of a sound with minimal information value when presented to animals in a way that simulates important features of industrial sound environment?

1. *Short-term reactions.* The tail of the rat has proved suitable for studies on the peripheral vascular reaction to sound (Borg, 1977). A summary of results from a series of studies is given in Fig. 1. The peripheral vasoconstriction increases as a function of the intensity of a noise burst. This increase begins right from the hearing threshold. The significant observation is that it is the final level reached during at least 0.1 s, and not the magnitude of the increase of the sound above the background level, that is decisive for the degree of vasoconstriction (Fig. 1 A, B, C). Only when the change is less than 5–10 dB, does the reaction fade. A temporal integration takes place such that sound bursts of 10–50 ms duration are less effective in eliciting a response than are longer bursts (Borg, 1978*a*). The way in which the rate of rise to the final level influences the response is illustrated in Fig. 1 E. It is seen that a slow rise (rise time 1 s) will damp the reaction (Borg, 1978*b*). When the animal has become habituated to a continuous sound after about 1 h, a brief pause in the sound will elicit a vasoconstriction (Borg, 1978*c*) which may be more pronounced than that elicited by a correspondingly long noise burst (Fig. 1 D). It is thus a combination of the level and the temporal pattern of the sound that is decisive for the reaction. The role of frequency has yet to be investigated.

Habituation takes place rapidly when the sound is continuous. Intermittently presented noise bursts, on the other hand, elicit responses throughout an entire day, and the habituation on the subsequent days will be only minimal. A gradual habituation may be observed after several experimental sessions, and this habituation is most marked at low sound intensities.

In conclusion, non auditory effects of sound depend on the acoustic properties of the signal. Habituation is rapid under continuous stimulus conditions, but under intermittent stimulus conditions, acute reactions may be elicited during several days, at least.

2. *Long term effects.* On the basis of the acute experiments, one would expect that permanent alterations in physiological homeostasis—and, in turn, effects on longevity and health—might occur. Evidence exists that ex

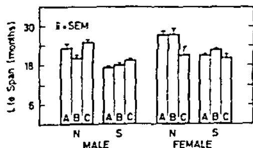


Fig 2 Average life-span of normotensive (N) and spontaneously hypertensive (*S*) rats in three acoustic environments (A) Control background <50 dB(A) (B) 85 dB SPL (C) 105 dB SPL In the noisy environments frequency modulated chopped narrow band noise was presented from 8 p m to 8 a m for more than 2 years (from Borg et al 1978)

tremely intermittent sounds presented over at least a few months do lead to a rise in blood pressure (Buckley & Smookler, 1970) Such experiments have used sound alone, or in conjunction with other sensory stimuli, in order to produce neurogenic hypertension They thus form a poor basis for evaluating the effects of the more continuous and realistic types of noise encountered in most industrial situations By realistic is meant that the sound may be steadily variable but can be considered in the context of a longer period of time The sound should also be presented while the animals are usually most active, i e at night One crucial question is whether sounds presented according to a realistic schedule can alter homeostasis and cause disease It is still of importance to separate the effects of sound itself from those effects associated with sound as a warning signal

A long term study on the general effects of a realistic sound environment has been completed recently In this study the effect of irrelevant, meaningless sound on several physiological parameters was investigated in rats These included blood pressure life span and incidence of disease Two groups of rats were used, normotensive rats and a risk group spontaneously hypertensive animals The rat was chosen because of its relatively short life span, the availability of normal animals as well as a strain of high risk animals and the possibility it offers for non invasive blood pressure measurements From about 3 months of age, the animals lived in one of three sound-isolated environments Environment A was used as control no external noise Environment B 85 dB SPL (sound pressure level re 20 μPa) Environment C 105 dB SPL sound The animals were exposed from 8 p m to 8 a m with a total of 2 hours of random pauses (rats are most active during the night) The sound consisted of a 1640 Hz noise-band sweeping from 3 to 30 kHz at a rate of 0.5 Hz and chopped at 0 5 Hz with a duty cycle of 50% The noise, then, was of a continuous nature but always varying in its details Blood pressure was measured repeatedly with a non-invasive 'tail cuff' technique In addition body weight, water consumption and life span were determined individually At autopsy organ weights, cause of death and diseases were assessed

The results showed that blood pressure was not affected by exposure, either in normotensive or in spontaneously hypertensive rats (Borg & Moller, 1978) Nor did the other physiological parameters differ between the different sound environments Fig 2 shows the average lifespan for normotensive and spon-

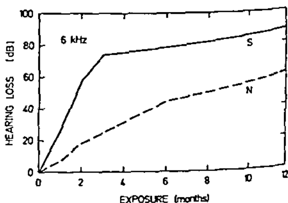


Fig 3 Average hearing loss at 6 kHz (measured threshold – mean threshold for young non-exposed rats) as a function of exposure time in normotensive (N) and spontaneously hypertensive (*S*) animals The *S* and N rats exposed for 12 months were of the same age The *S* rats exposed 1–3 months were in the high age range whereas the corresponding *N* rats were young

taneously hypertensive rats in environments A, B and C In all groups, many animals had renal diseases, gastric ulcers, and cardiovascular lesions It is true that a detailed analysis of the disease panorama would demand a much larger number of animals, but it is clear from the present data that no great differences exist The average number of diseases signs diagnosed per animal in different groups at autopsy did not differ significantly (Borg et al, 1978)

In conclusion, irrelevant, meaningless but realistic" sound of the type used in the present study does not interfere with health or bring about chronic physiological changes in rats In order to apply these findings to man, one has to remember that sounds are often not as irrelevant or meaningless as for the rats in these experiments For man, e g in a factory, sounds often contain warning signals and interfere with verbal communication Even if a warning signal, generated e g by a machine that is not functioning properly, is decreased by 20 dB, it still carries the same information It will most likely elicit the same reactions as long as it is heard With regard to the issue of controlling noise, the present findings seem to indicate that it is not noise itself that is crucial but rather the riskful processes about which the noise conveys information

B *Physiological aspects of individual variability in susceptibility to sound induced hearing loss*

Sound presented according to a realistic' schedule did not cause a rise in blood pressure in rats In order to determine whether this lack of effect was due to a profound hearing loss produced by the acoustic environment, hearing thresholds were determined for all rats with a behavioral technique (conditioned suppression) after one year of exposure For the rats exposed to 85 dB noise, hearing loss was found to be 10–15 dB, for normotensive as well as for spontaneously hypertensive animals Regarding rats exposed to 105 dB noise, the normotensive animals had a hearing loss of less than 40 dB, whereas the spontaneously hypertensive ones had a profound hearing loss, at least 60 dB (Hearing loss is the difference in threshold between exposed and non exposed animals of the same age) An interesting observation was thus made noise does not cause hypertension, but hypertensive animals are more susceptible to noise-induced hearing loss A further analysis of this observation is now under way and a preliminary summary of the findings is presented in Fig 3 It is seen that the spontaneously hypertensive animals undergo a faster deterioration in hearing threshold as a function of exposure time than do the normotensive ones The interpretation of the findings shown in Fig 3 is, however, not yet quite definite, since the spontaneously hypertensive rats tested so far have been comparatively old even though their exposure has lasted for only a few months The normotensive rats have been younger Two possible causes of individual variability in susceptibility to hearing loss thus may operate, age and hypertension (or factors genetically coupled to the latter phenomenon)

Although variation in inner ear physiology is of great importance for individual susceptibility, the sound transmission system is capable of introducing a considerable variability in the actual acoustic input to the inner ear structures If the head and ear canal resonances and the middle ear impedance matching create a 10 dB higher sound level, at e g 3 kHz, in one subject than in another, it is hardly noticeable at threshold or in an ordinary sound environment At harmful levels, however, it may be of the utmost importance If sound transformation in a subject is, say, 10 dB above the average, he is likely to suffer considerably more injury than most other persons working in the same environment

Surprisingly, the range of individual variability brought about by the head and ear canal resonances, the middle ear impedance matching, the middle ear muscle threshold, and the rate of decay in the reflex response may each

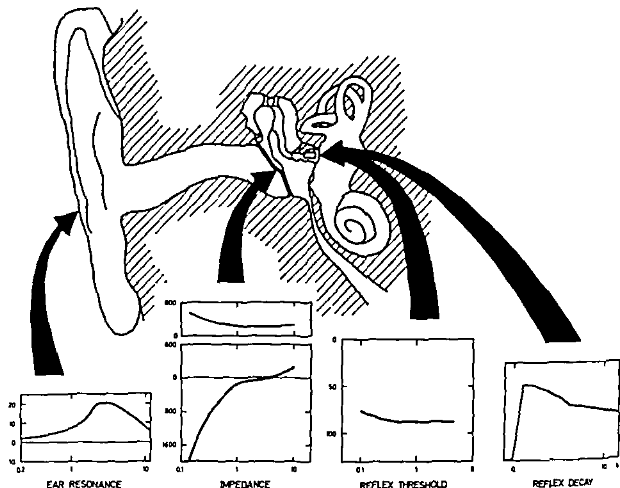


Fig 4 Schematic drawing of the ear together with a mplified outline of the physiological properties of the n parts of the sound conduction system Heavy lines indicate averages and the shaded area shows roughly normal variation Resonance of head pinna and ear canal (dB on ordinate) Middle ear impedance (acoustic Ohms on ordinate) Reflex threshold (dB HL on ordinate) Reflex decay (relative response amplitude on ordinate)

contribute to a 10 dB variation in the actual sound input to the ear, at least in certain frequency ranges A schematic ouline of these features is given in Fig 4 It is of couse a mistake to believe that these variations can be added to one another in a simple way They may in fact compensate for each other, and we have very little information as to what they really mean for individual susceptibility These properties have been largely overlooked, despite their being accessible for direct measurement, in contrast to most inner ear properties An interesting fact in this connection is that hearing thresholds, e g at 3 kHz, fall within ±12 dB for 94% of young persons with "normal" hearing (Wheeler & Dickson, 1952) There is no reason to believe that this variability is due entirely to inner ear and nervous system properties In addition, it is obvious that it is not the average variability that is of interest but rather the maximum range of variability, which can be even greater than 20 dB

In summary, no evidence has been found of permanent, harmful effects of meaningless, irrelevant sound on non-auditory body functions of rats On the other hand, there is substantial evidence that physiological factors are involved in individual susceptibility to hearing loss

ZUSAMMENFASSUNG

Fur unterschiedliche Gerausche wurde eine Reihe von kurzzeitigen und langzeitigen physiologischen Effekten auf nichtauditive Körperfunktionen untersucht Die Ergebnisse können wie folgt zusammengefaßt werden Die Kurzzeiteffekte sind weitgehend durch die unterschiedlichen akustischen Parameter der Geräusche bedingt Bei Dauersignalen zeigt sich eine rasche Habituierung, bei unterbrochenen Signalen dagegen erfolgt sie langsam Ratten wurden während ihrer ganzen Lebenszeit irrelevanten, d h sinnlosen Gerauschen als Substitut einer industriellen Larmumgebung ausgesetzt, diese Dauerbeschallung zeigte jedoch keine Einwirkung auf den Blutdruck, die Lebensdauer oder die Haufigkeit von Krankheiten Mit Hinblick auf traumatische Schalleinwirkung auf das Innenohr konnte festgestellt werden, daß spontan hypertensive Ratten betrachtlich empfindlicher waren als normotensive Daraus kann geschlossen werden, daß Schallreize an sich keine Hypertension hervorrufen, daß jedoch die Empfindlichkeit für Schädigungen des Innenohrs bei hypertensiven Individuen großer ist Abschließend wird die individuelle physiologische Variation des Schalleitungssystems diskutiert ein Faktor, der in der individuellen Unterschiedlichkeit von larmbedingten Hörverlusten von Interesse sein durfte

ACKNOWLEDGEMENT

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DIAGNOSIS AND TREATMENT OF OCCUPATIONAL NOISE-INDUCED HEARING LOSS

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The audiometric configuration of noise-induced hearing loss (NIHL) is well-known Characteristically we find a symmetric high tone loss with a maximum at 4 and 6 kHz It appears that frequencies below 1 5 kHz are not greatly influenced by noise, not even after many years of exposure This is probably partly due to protection from the stapedius reflex The earliest audiometric changes can often be found at 6 kHz Formerly, this frequency was not tested and the early detection of slight NIHL was consequently delayed Another characteristic finding of NIHL is recruitment which can be shown by decreased stapedius reflex sensation levels or by decreased excursion width in Bekesy audiometry

In general we find an agreement between the patient s noise exposure and the resultant degree of hearing loss It should be remembered, however, that the pure tone audiogram shows the result of all accumulated noise exposure from childhood, adolescence, military service and leisure activities in addition to occupational noise exposure Typically, because of the slow development of NIHL and our poor memory, the extent to which different factors contribute to the resulting hearing loss are difficult to evaluate

Deviations from the typical audiogram are often seen Some people show unusual noise resistance These patients have what we suggest calling "stone ears Contrarily we quite frequently encounter patients with a severe hearing loss in relation to their noise exposure Often we feel that genetic factors contribute to such impairments However typically it is impossible to tell how much each of these factors, genetic deficiency and noise exposure contributes to the patient's severe hearing loss His hearing at this age in fact could have been the same without any noise exposure However, it would seem desirable to be able to identify the "glass ears" and the progressive hearing loss of such a patient at an early age In this case such identification should have resulted in the patient being advised to avoid occupations with noise exposure

Occupational NIHL can be divided into four kinds (*a*) that from continuous noise, (*b*) from impulse noise, (*c*) from a combination of (*a*) and (*b*), and (*d*) that from acoustic trauma Similarities and differences of the three basic types of NIHL are shown in Table I

Many different factors have been claimed to increase the risk for noise-induced hearing loss These are

great intensity	genetic and/or individual susceptibility
long duration	increased blood lipids
high frequencies	hypertension?
pure tones	male sex?
impulses	
simultaneous vibrations	

Both intensity and duration contribute in a fairly similar way to the development of NIHL It is also well known that high frequencies pure tones, and impulses increase the risk for occupational hearing loss The contribution of factors such as increased blood lipids, hypertension, and genetic differences between the male and female are much more debatable The only therapeutic measures available are hearing aids, technical means and audio-educational supporting methods such as

Table I *Noise induced hearing loss—differential diagnosis*

	Gradual onset		Acute onset
	Continuous	Impulse	Acoustic truma
Occurrence	Industrial	Industrial military leisure activities	Industrial military leisure activities
Incidence	Common	Less common	Uncommon
Development	Very slow	More rapid	Acute
Subjective symptoms	Late	Earlier	Immediate
Symmetry	Yes	Yes or no	Often no
Frequencies affected	1 5–8 kHz	2–8 kHz	Any–all
Order kHz	6 4 8 3 2 or 4 6 8 3 2	6 4 3 8 2 or 6 4 8 3 2	
Audiometric configuration	Sensorineural high tone	Sensorineural steep high tone	Sensorineural high tone and other
Mechanism	Metabolic and/or oxygen deficiency	Mechanical injury of organ of Corti	Inner ear membrane breaks? Hemorrhage? Mechanical
Therapy	*Prophylaxis only* (Aural rehabilitation)	*Prophylaxis only* (Aural rehabilitation)	(*Prophylaxis*) Low molecular dextran? (Aural rehabilitation)

hearing aid orientation, auditory training and speech reading

The components of prevention can be termed *the hearing conservation program* which consists of

hearing conservation committee
sound level measurements including noise dosimetry
pure tone audiometry including controls
ear protection including information
technical noise abatement

It is our belief that the hearing conservation committee must be responsible for all actions in the program Regrettably, such a committee *is often the missing link in the chain of hearing* conservation Sound level measurements, including noise dosimetry, constitute the basis for establishing the existence of injurious noise levels Such measurements are performed in many modern industries today An equally important measure is audiometry with regular follow up testing in order to identify NIHL as early as possible Audiometry also has the advantage of giving information on individual susceptibility differences to noise and on satisfaction afforded by the adopted hearing protection program There are *many different ear protectors available today It is desirable* to have a supply of different kinds so that each worker can choose the one most to his liking otherwise he will be less inclined to use hearing protectors It is obviously better to accomplish a high usage of ear protection which is readily accepted but perhaps a little less effective, than reluctant and insufficient use of generally issued ear protectors We feel that a wealth of information is needed concerning these matters Technical noise abatement is, in the long run, the most important factor, but it is also the most expensive There is today a world wide active technology, both for decreasing the noise at the source, *i e* making less noisy machines, and for adopting more efficient sound protective materials

The characteristics of NIHL are, in brief

high incidence
slow development
large individual variations in susceptibility/resistance
hearing loss irreversible
hearing loss goes unnoticed by exposed worker until advanced
hearing loss can be *measured and controlled*
prevention is the only therapy

Some of these characteristics should alert those responsible for health and safety of the workers, others can be used to advantage in the prevention of hearing loss

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PSYCHOLOGICAL EFFECTS OF NOISE

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Abstract The most frequent harmful effect of noise is its irritating psychological influence on the individual This influence has been measured in laboratory situations and in daily life by careful sociological interviews of the inhabitants in noisy and in quiet residential areas Statistically significant differences were demonstrated between the two areas regarding consumption of soporifics tranquillizers isolation against noise etc

Hearing is of paramount importance for communication and is moreover a warning system, which functions around the clock irrespective of light or darkness, whether we are awake or sleeping Connections from the central hearing pathways to the autonomous nerve system prepare the organism for activity at the slightest sign of danger Changes have been demonstrated in blood pressure, pulse rate, vasoconstriction of the peripheral vessels, pattern of respiration etc The psychological effects of noise differ from person to person and, in one and the same person dependent on the hour, the character of the noise, and individual variables The most common harmful effect of noise is the psychological irritation This parameter is difficult to measure in comparison with its damage to hearing, which is more rare The noise-induced hearing impairment gives rise to difficulties in communication, which is a stressfactor quite different from loss of a finger, for instance The data obtained by pure-tone audiometry and speech discrimination tests do not indicate the true picture of a person's hearing handicap in the way it is experienced by himself Ewertsen & Birk Nielsen (1972) have proposed a standardized scale for self-assessment of the handicap, consisting of 21 questions with which a patient's bias to answer in an affirmative or a negative way is balanced out and scored This gives a social hearing handicap index (SHI) where 100% means maximum handicap The index is [illegible]lated to the speech reception threshold, a[illegible] wide deviations of the SHI are seen, from 5 up to 100%, even in cases with a relatively good SRT This means that patients with identical audiometric characteristics get along quite differently in daily life

The harmful, irritating effect of noise gen[illegible]erated by the functions of the community ([illegible]ciocusis) has been investigated by Rossi et [illegible] (1976) In laboratory situations, normal hea[illegible]ing subjects have been exposed to vario[illegible] kinds of recorded traffic noise Short tempo[illegible]rary threshold shifts and changes in pattern of sleep could be demonstrated However, laboratory tests do not correspond to daily life where individuals are exposed to noise day and night The safest evaluation is secured by a careful sociologic examination of the [illegible]habitants in noisy and in quiet residenti[illegible] areas Relster (1975) has interviewed 96[illegible] housewives with regard to the influence o[illegible] traffic noise on their physical and psycholog[illegible]

Table I *The influence of noise on behaviour*

	Noisy area (n=477) (%)	Quiet area (n=483) (%)
Medical consultations because of psychic problems	19	12
Use of sleeping medicine	12	6
Tranquilizers	25	17
Mental hospitals	4	2
Closed windows	64	9
Isolation against noise	18	2
Position of rooms changed	20	2

cal well being Half of the women were living in quiet areas where the noise level fluctuated between 51 and 63 dB(A) over 24 hours, while the level fluctuated between 69 and 78 dB(A) in the noisy areas Relster defines noise as sounds with a negative influence on a man's physical and psychic well being, including change of behaviour and way of life in a direction experienced as negative by the individual The changes of behaviour are given in Table I The differences for all the indicated parameters are statistically significant The need to live with closed windows is dramatically greater in the noisy area (64% against only 9% in the quiet area)

The only way to solve the problems of comfort is to pass certain new laws concerning the planning of cities, motor-roads, railways and airports in order to separate residential areas from industrial areas and noisy transport

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NOISE-INDUCED HEARING LOSS AND THE COMPREHENSION OF SPEECH IN NOISE

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Abstract Subjects suffering from noise induced hearing loss (NIHL) with or without hearing loss at 2 kHz were examined by speech audiometry with a three-digit test bisyllabic and monosyllabic PB word lists in silence and in USASI noise with a masking effect of 44 dB and 68 dB at 1 kHz Subjects with up to 20 dB hearing loss at 2 kHz had almost the same speech comprehension in noise as normal hearing subjects Subjects with hearing loss greater than 20 dB at 2 kHz had increasing discrimination loss at increasing noise levels They also needed a signal to-noise ratio better than normal hearing persons would yield at the noise levels used The [illegible]

for the other half in noise

Noise-induced hearing loss (NIHL) affects first and foremost the frequency area 4–6 Hz, usually with a maximum at 4 kHz As hearing loss worsens it spreads to lower equencies and even affects 2 kHz which is the frequency of greatest importance to speech comprehension Even though the ability to perceive speech via the telephone or in face-to face conversation may be well preserved, subjects with high tone hearing loss have early on some difficulty in understanding speech in noisy conditions (Anianson, 1974, Kuzniar, 1973)

As the assessment of the hearing threshold for pure tones is both simple and reliable, numerous methods have been proposed for calculation of the hearing loss for speech in silent conditions (Alberti et al , 1976) and in noise (Pickett & Pollack 1958) on the basis of the hearing loss for pure tones It would seem however, that there is no formula which with satisfactory confidence can predict the discrimination and hearing loss for speech of an individual ear on the basis of the hearing threshold for pure tones (Harris 1965 Meyers & Angermeier, 1972) One and the same loss for pure tones may result in differing hearing loss for speech (Quist Hanssen & Steen, 1960) Hence it seems necessary to assess discrimination and hearing loss for speech directly by speech audiometry

In Norway the evaluation of the hearing disablement is based almost exclusively on the results of pure tone and speech audiometry in a silent room If there is a discrimination loss the hearing loss for speech is corrected through the hearing index table published by Frenckner et al (1958) The percentage hearing disablement caused by assessed or corrected hearing loss for speech is given in tables of the National Insurance System of Norway based on the computed binaural hearing loss for speech

To subjects with NIHL, this procedure leads in the majority of cases to a very mild hearing disablement which does not reflect the difficulties they experience when trying to comprehend speech in noisy everyday conditions (Anianson, 1974, Klockhoff & Liden 1974) We therefore need an examination procedure better than speech audiometry in silence which can predict the speech hearing capacity of subjects with NIHL in everyday noisy conditions At the Institute of Audiology of the National Hospital of Norway a background "USASI noise' has been used Its electrical spectrum appears from Fig 1 The sound spectrum of 65 dBSPL ordinary room noise and its computed masking effect

appears from Fig 2A Fig 2B shows that the observed masking by 55 dBA USASI noise conforms closely to the computed masking of 65 dBSPL room noise

For speech audiometry we used Quist-Hanssen's word lists (1970) (a three-digit test and PB lists of bisyllabic and of monosyllabic words) and USASI noise on tapes

Two noise levels were used, giving 62 dBSPL (58 dBA) and 82 dBSPL (78 dBA) respectively in a 6 cc coupler

In normal hearing subjects the noise levels produced 44 dB and 68 dB hearing threshold level at 1 kHz and masking audiograms as shown in Fig 3

The material consists of 20 subjects from 35 to 83 years of age, averaging 57 years, 2 women and 18 men Sensorineural high tone hearing loss was present in 36 ears, in 26 ears of characteristic NIHL type with better hearing at 8 kHz than at 3–6 kHz

Seven ears in which the hearing loss at 2 kHz did not exceed 20 dB are labelled NIHL II degree, 29 ears whose hearing loss exceeded 20 dB at 2 kHz are labelled NIHL III degree [1]

Eight normal ears in young adults served as controls The mean hearing threshold level in the subjects with normal hearing II or III degree NIHL appears from Fig 4 The results from speech audiometry in silence and two levels of USASI noise are shown in Table I

The hearing threshold levels of the normal ears in silence are in agreement with previous findings The common hearing threshold level for the digit test, the bisyllabic and monosyllabic PB word lists is the intended result of the calibration of the speech audiometry material (Quist Hanssen, 1970)

In all three listening conditions, subjects with NIHL scored better in the digit test than in the bisyllabic and monosyllabic word tests The difference is greater in silence than in noise, as in silence subjects with NIHL benefit from their preserved hearing in the low and middle frequency areas for the understanding of the digits which, for statistical

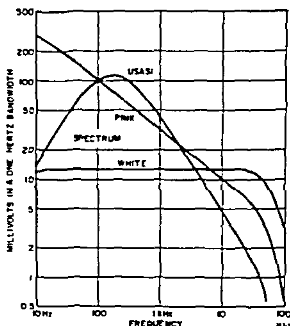


Fig 1 Voltage spectra for three different masking noises

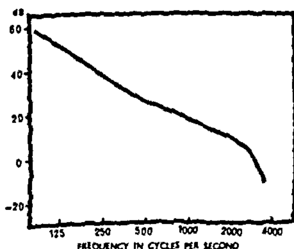
reasons, require only a small amount of information For the same reason, normal-hearing subjects show a lower hearing threshold level for digits than for bisyllabic and monosyllabic words in noise

In USASI noise of 58 dBA the subjects with II degree NIHL need a signal to-noise ratio (S/N ratio) from 0 to 5 dB better, and those with III degree NIHL from 5 to 16 dB better than what normal hearing subjects require

In USASI noise of 78 dBA, subjects with II degree NIHL need a S/N ratio which does not differ from that of normal hearing subjects, while those with III degree NIHL need an S/N ratio 6–11 dB better, as shown in Table II

Although the need for a better S/N ratio decreases with increasing SPL of the noise, increasing discrimination loss (which appears from Table III) reduces the comprehension

[1] II but with additional hearing loss exceeding 20 dB at 2 kHz



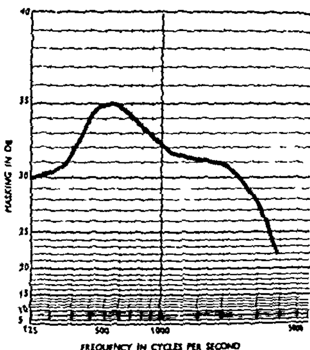


Fig 2A Sound spectrum and computed masking from 65 dBSPL ordinary room noise

of speech For satisfactory speech perception, subjects with NIHL *must* get the *S/N* ratio they need

Which *S/N* ratio will normal-hearing speakers offer the listener in a background noise? In investigations (by G Flottorp, Sv Quist-Hanssen and A Sundby) speaker and listener sat 2 5 m apart in the USASI noise in a non-reverberant room The speaker spoke short sentences which the listener should understand and repeat As in an everyday face to face conversation the speaker could check that he was understood Lip reading was not used The results are given in Fig 5 which shows that the *S/N* ratio decreases with increasing SPL of the noise, as previously dem

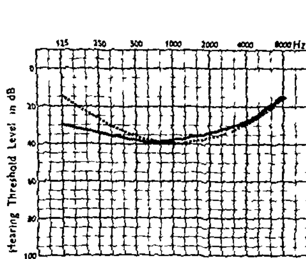


Fig 2B Computed masking from 65 dBSPL ordinary room noise — and observed masking from 55 dBA USASI noise

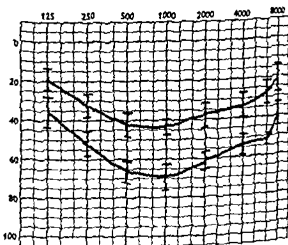


Fig 3 Masking audiogram from USASI noise of 62 dBSPL (58 dBA) and 82 dBSPL (78 DBA) Mean hearing threshold level and standard deviation for pure tones in 8 normal ears

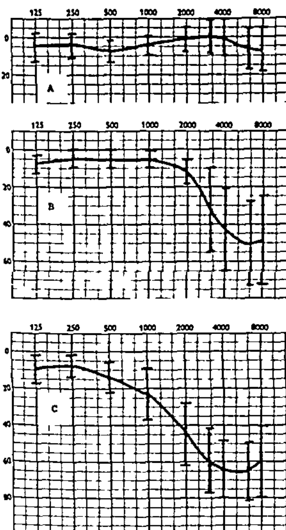


Fig 4 Mean hearing threshold level for pure tones in (*A*) normal ears (no 8) (*B*) noise induced hearing loss II degree (no 7) (*C*) noise induced hearing loss III degree (no 29)

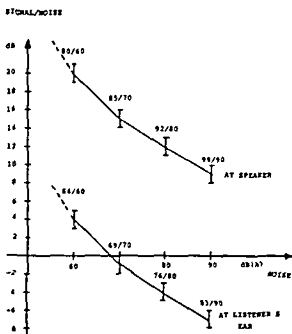


Fig 5 Signal to-noise ratios obtained by using sentences in USASI noise 2 5 m distance between speaker and listener and no lip-reading

onstrated by Kryter (1965) and Picket & Pollack (1958) Already at 60 dBSPL USASI noise the mean S/N ratio is reduced to 4 dB at the listener's ear and at 65–70 dBSPL to 0 dB

At −1 dB S/N ratio the normally hearing subjects perceived all the sentences and at −4 dB, 80% of them, thus agreeing with observations of Flanagan (1965) and Pickett (1956) However, large individual differences

Table I *Mean speech reception threshold level dB re 20 μPa in silence and in noise for subjects with normal hearing and II and III degree NIHL*

Di = digits Bi = bisyllabic Mo = monosyllabic words

NIHL	Silence			58 dBA USASI noise			78 dBA USASI noise			N
	Di	Bi	Mo	Di	Bi	Mo	Di	Bi	Mo	
0	32± 5	33±2	34± 3	67±2	72±3	70±3	86±2	93±3	90±2	8
II	37+ 5	44±4	45± 7	67±4	71±4	75±4	84±3	93±7	94±3	7
III	46+10	57+9	66±11	72±5	81±7	86±8	92±5	99±5	101±7	2[illegible]

Table II *Difference in dB between speech reception threshold level in subjects with normal hearing II and III degree NIHL in silence and in USASI noise*

Di = digits Bi = bisyllaabic Mo = monosyllabic words

NIHL	Silence			58 dBA USASI noise			78 dBA USASI noise		
	Di	Bi	Mo	Di	Bi	Mo	Di	Bi	Mo
II	5± 5	11±4	11± 6	0±4	−1±4	5± 4	−2±3	0±7	4±4
III	14±10	24±9	22±11	5±5	9±7	16± 8	6±5	6±5	11±7

are observed in the speech SPL given by dif ferent speakers in the same noise (Pickett, 1956) In noise up to 85 dBA one should, how ever, expect normal hearing subjects to yield a S/N ratio at least 5 dB above the critical ratio for normal hearing subjects, and in many cases even 10 dB, though decreasing with increasing SPL of the noise (Kryter, 1965)

Table II shows that subjects with II degree NIHL at both the USASI noise levels used may expect a signal to noise ratio which per- mits them to comprehend speech For the majority of the subjects with III degree NIHL the S/N ratio presented will not be satisfactory and will hinder or prevent speech perception

At SPL above 78 dB the intelligibility of speech in noise is not only reduced, but is so to an increasing extent with increasing SPL, despite a constant S/N ratio (Pickett & Pollack 1958) Even if the actual S/N ratio were to satisfy the needs of the hearing impaired for speech comprehension, the *increasing SPL of the noise would cause an increasing discrimination loss and thereby* reduce speech perception (see Table III)

Table III *Percentage discrimination loss (PB monosyllabic words) in silence and in noise in subjects with II and III degree noise induced hearing loss*

NIHL	Silence	58 dBA USASI noise	78 dBA USASI noise
II	0± 0	4 3 ± 11	10 ± 15
III	9±13	13 3 ± 15	28 1+24

Subjects with high tone hearing loss benefit from lip reading in noise just as much as in silence (Klockhoff & Liden 1974) The ability to lipread varies from person to person and the benefit obtained depends on good sight and good lighting Lip reading should there fore not be taken into account in the evalua tion of hearing disablement The advantage of binaural (dichotic) hearing in everyday life is *fairly uniformally evaluated (Lierle 1961* Alberti et al , 1976) 1/5 for the bad ear and 4/5 for the good ear may be a reasonable suggestion

Scarcely one half of the speech we com prehend is presented to us clearly and under favourable listening conditions (Harris 1965) With NIHL, the hearing loss for speech as sessed by speech audiometry in silence should count for no more than one half of the hearing disablement The other half should be based on the result of speech audiometry against a background noise representative of everyday noise (such as *USASI) of about 58 dBSPL and the required S/N ratio and discrimination* ca pacity must be decisive factors

The results of these examinations should however be compared with the hard of hear ing person s own appraisal of his hearing as emerges from interview according to stand ardized questionnaire

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MILITARY AUDIOLOGICAL ASPECTS IN NOISE-INDUCED HEARING LOSSES

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Abstract The sound pressure of a single shot ranges from about 165 up to 190 dB depending on the type of weapon In general most shooting practice is carried out from a sheltered shooting gallery Groupshooting and multiple reverberations from sheltered galleries maximize the acoustic strain Clinically the acoustic trauma can be considered to be a combination of both acute and chronic noise injury because very intense impulse noise such as gun fire can injure the inner ear both mechanically and by mean of disturbances in metabolism When the ears are not protected acoustic trauma develops in about 10% of military conscripts and 60% of regular army personnel

Gunfire can be described in physical terms as intensely strong impulse noise The sound pressure of a single shot measured at the level of the ear of the marksman ranges from about 165–190 dB, depending on the weapon (Table I) Most shooting by both military conscripts and regular army personnel is carried out from a sheltered shooting gallery It is typical of shooting that within a relatively short period of time several series of shots come from several weapons Numerous reverberations echo from the walls floor and ceiling of the shelter, causing the duration of the gunfire noise to increase many fold It can be stated that shooting in large groups and from sheltered galleries maximizes the acoustic strain of shooting Noise loads of over 120 dB/h can be registered with a dose meter in conjunction with routine shooting excercises (Table II) Sound pressures of this magnitude injure the inner ear both mechanically and by mean of disturbances in metabolism

Clinically the hearing defect caused by gun fire, acoustic trauma can be considered to be a combination of acute and chronic noise injury The hearing loss begins and develops rapidly Only a few shots—or at times even a single shot—are enough to cause a measurable hearing loss Although there are initially major temporary threshold shifts, such defects are usually accompanied by permanent hearing losses as well This is explained on the basis

Table I *Intensity of reports produced by different weapons*

Weapon	mmHg	dB
Pistol	37 5	168 0
Rifle	50 7	170 6
Automatic rifle	54 0	171 1
81 mm mortar	64 0	172 6
120 mm mortar	83 0	174 9
20 mm anti aircraft rifle	88 0	175 4
Recoilless antitank gun (55 S 55)	784	185 6
Field cannon 105 H/61 37	377	188 0

Table II *Noise dose measurement of group shooting from shooting gallery*

Nearest distance at which the noise dose could be measured was 4 metres behind the marksmen as the maximum sensitivity of the noise dose meter was only 140 dB

Equipment

Bruel & Kjær Precision impulse sound level meter type 2209

Bruel & Kjær Noise dose meter type 4473

Results

[illegible]

Firing (=I+II+III) carried out within 0 749 h and total noise dose at that time was 118 dB (A) (measured 4 metres behind the marksmen)

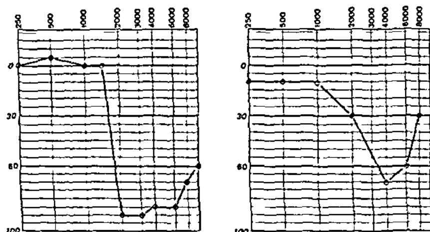


Fig 1 Left a typical chronic acoustic trauma caused by gunfire *Right* ordinary noise-induced hearing loss

of both the mechanical effects as well as the disturbances in the metabolism exerted by the very intense sound energy Initially in acute shooting or explosive hearing injuries there is a flat inner ear defect which gradually disappears within several weeks or months, but usually a permanent, clear C_5 dip remains In chronic acoustic trauma the extent of the temporary threshold shift is less marked and recovery from it is more rapid A typical audiogram is a sharp C_5 dip (Fig 1), which becomes deeper and wider as the sound strain caused by the shooting continues The dip begins at a frequency of 6000 Hz twice as often as it begins at 4000 Hz This trauma develops very rapidly in comparison with normal noise injury According to some studies those who shoot much and frequently develop within a year or two a permanent hearing defect which either ceases to progress, or progresses slowly

When the ears are not protected, AT develops in about 10% of military conscripts Correspondingly acoustic trauma develops in 60% of regular army personnel (Table III) The number of hearing defects decreases significantly when the ears are protected An analysis of regular army personnel demonstrated that the speech reception threshold was 10 dB or better in 80% and worse than 30 dB in less than 2 5% Speech discrimination is better than 90% in 93% when tested under quiet conditions (Table IV) Hearing defects developing in conscripts are usually slight dips, without significance, occurring at 4000 to 6000 Hz

On the basis of preliminary observations it seems likely that the majority of hearing changes in servicemen can be prevented with the use of ear protectors and by changing the method of shooting

Table III *Hearing status of Finnish regular army personnel (1963)*

Normal hearing	33 2%
Acoustic trauma	57 2%
Other lesions of ear	9 6%
(total 422 persons)	

Table IV *Speech audiometric findings in acoustic trauma*

Speech threshold (dB)	
<10	80 5%
11–20	15 3%
21–30	1 7%
>30	2 5%
(total 355)	
Discrimination	
>90%	93 4%
90–80%	4 3%
<80%	2 3%
(total 355)	

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NOISE-INDUCED HEARING LOSSES

Can They Be Explained by Basilar Membrane Movement?

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An understanding of noise induced hearing losses requires a knowledge of the movement of the basilar membrane and the resulting hair cell strain, for a given noise spectrum However, this knowledge is at present inadequate The following analysis is based on Mossbauer effect measurements of the basilar membrane movement in seven human temporal bone preparations (prepared 8–24 hours post mortem) The movement of a small radioactive source placed on the basilar membrane is determined after measuring the velocity dependent transmission of the radiation from the source through a suitable absorber This technique allows measurement of very small movements

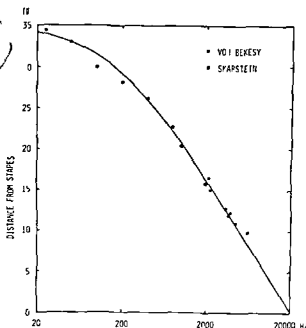


Fig 1 Distance from stapes to the place of maximum displacement as function of frequency The data points measured as well as those of von Bekesy (1960) fit the curve given by Greenwood s formula (1961)

Analysis

Pure tone stimulation gives maximum basilar membrane deflection at a characteristic place The corresponding frequency may be called the characteristic frequency or the place frequency When the frequency increases the characteristic place will move from helicotrema towards the stapes, as is shown in Fig 1

Frequency responses for basilar membrane displacement have been measured at one basilar membrane position in each of seven temporal bones The measuring positions were in the distance range 9 9–16 5 mm from the oval window, corresponding to place frequencies

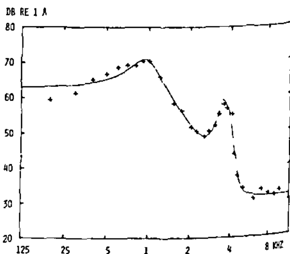


Fig 2 Displacement responses at the 3 6 kHz place 12 0 mm from the stapes SPL at eardrum 120 dB

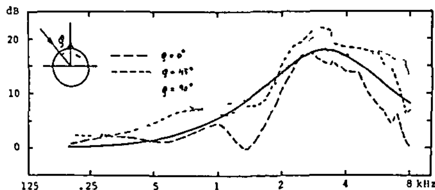


Fig 3 Level increase ... diffuse field to ear ... approximated f ... Ross data for ... of incidence ... Ross 1976)

2 2–6 2 kHz The measured response at the 3 6 kHz place is shown in Fig 2

It is well known that the hair cells in the 4–6 kHz site range are the most vulnerable to noise damage This seems to contradict the fact that usually the damaging noise has most of its energy at lower frequencies, which has been regarded as an unexplained paradox Part of the explanation probably follows from Fig 2 Somewhat surprisingly, at the 3 6 kHz place, a constant sound pressure at the ear-drum will give rise to a larger displacement at 1 kHz than at the characteristic frequency 3 6 kHz Low frequency noise components will thus give substantial contributions to basilar membrane displacement and hair cell strain in the place range where the hair cel... vulnerable to noise damage

The sound pressure level at the ... larger than in the external ... level increase from diffuse field ... shown in Fig 3

By taking this level increase ... the displacement response ... ascribed to a constant sound ... external field This is done ... also shows the correspond...

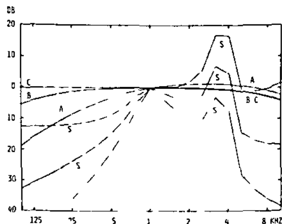


Fig 4 Displacement velocity and acceleration weighting curves near the 4 kHz site at the basilar membrane shown together with the standardized ... ing curves S – displacement $\dot{S}$ – veloc...



Fig 5 Cor...

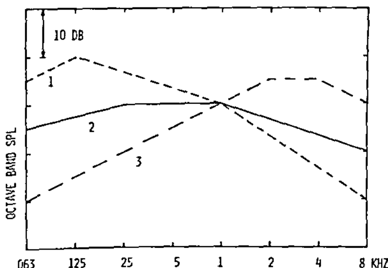


Fig 6 Examples of noise spectra *1* falling *2* medium and *3* rising

acceleration responses, together with the standardized A, B and C weighting curves

The responses are used as weighting curves to find the displacement, velocity, and acceleration levels at the 3 6 kHz place, when the external sound spectrum is given These levels may be compared with the ordinary A-, B- and C-weighted sound levels This is of interest because the standardized weighting curves A, B and C simulate the frequency weighting of the ear itself, in order to give correlation between weighted sound levels and ychoacoustic and physiological data

For 121 industrial noises the A-, B and C- I sound levels, and displacement-, velocity- and acceleration levels are compared in the scatter diagram shown in Fig 5

Of particular interest is the close correlation between displacement levels and B-weighted levels According to Burns & Robinson (1970), noise induced hearing losses correlate even better with B weighted levels than with A-weighted levels Consequently, the damage of at least the most sensitive outer hair cells seems to be determined by the basilar membrane *displacement* This conclusion is in agreement with what is generally accepted

As previously mentioned the seven displacement responses were measured in different ears and covered only a limited site range of the basilar membrane Despite the sparsity of the data, we have attempted to systematize and extrapolate their place dependency, in order to get an impression of how the displacement level varies along the membrane for known external noise spectra To find the corresponding mechanical hair cell strain the following assumptions are made

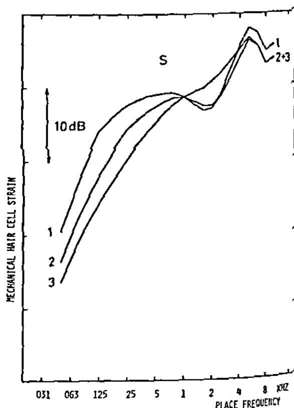


Fig 7 Mechanical hair cell strain levels for *1* falling *2* medium and *3* rising noise spectra

1. The hair cell strain is *proportional* to the basilar membrane displacement, the proportionality factor being *place dependent* due to the changing anatomical conditions
2. In order to detect sound, the hair cell strain at a characteristic frequency has to be larger than a *threshold* value, which for normal hearing is *place independent*, i e the same for all places along the basilar membrane

Mechanical strain of the hair cells means the mechanical parameter determining the electrochemical response of the hair cells

Only the results will be given here, all underlying details of the calculation procedure will be omitted For the rather different noise spectra in Fig 6, showing a rising, a medium, and a falling spectrum, the mechanical hair cell strain will vary along the basilar membrane as shown in Fig 7

The curves in Fig 7 show the interesting result that the hair cell strain is *maximum in the place range 4–6 kHz*, irrespective of the noise spectra, which on the basis of common experience is a very attractive consequence of the performed analysis

This analysis is, however, based on insufficient data of the basilar membrane movement for a given sound input to the ear It is therefore highly desirable, through measurement and inner ear modelling, to extend this knowledge in order to confirm or reject the explanation that the hair cells near the 4 kHz place are most vulnerable to noise damage simply because they usually will be exposed to the greatest mechanical strain

In any case, there seems to be no reason to doubt that low frequency components contribute substantially to the basilar membrane displacement and hair cell strain at this place

SUMMARY

The development of noise induced hearing losses must in some way be related to the basilar membrane movement Our analysis of this relationship is based on Mossbauer effect measurements of the basilar membrane movement in human temporal bone preparations At a single basilar membrane position in each of seven preparations the displacement frequency response was measured for a given sound pressure level at the ear drum The measurements covered the place range 2 2–6 2 kHz

Despite the inadequacy of the experimental data there seems to be no doubt that low frequency components contribute substantially to the displacement and mechanical strain of the hair cells near the 4 kHz location where the hair cells are known to be most vulnerable to noise damage In fact the analysis performed indicates that these cells will suffer the *greatest* mechanical strain almost irrespective of the spectrum shape of the stimulus noise

ZUSAMMENFASSUNG

Die Entwicklung von lärmverursachten Hörschaden muss in gewisser Weise in Zusammenhang mit der Bewegung der Basilarmembrane stehen Unsere Analyse von diesem Zusammenhang ist auf Mössbauer Effect Messungen der Bewegungsausschlage der Basilarmembrane in humanen Schläfenbeinpräparaten basiert An einer einzigen Stelle in jedem von sieben Präparaten wurde die Frequenzkurve der Amplitude für einen gegebenen Lautstärkepegel beim Trommelfell gemessen Die Messungen umfassten den Bereich für 2 2–6 2 kHz

Trotz der mangelhaften experimentellen Daten besteht daruber kein Zweifel dass niedrige Frequenzkomponenten in bedeutendem Grad zu der Bewegung und mechanishen Belastung der Haarzellen im 4 kHz Bereich beitragen wo die Haarzellen am meisten vor Lärmschäden verwundbar sind Ja in der Tat deutet die Analyse darauf hin dass eben diese Haarzellen der *grössten* mechanischen Belastung ausgesetzt werden fast unabhängig von der spectralen Zusammensetzung des Lärmstimulus

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NOISE-INDUCED HEARING LOSS IN DANISH BREWERY WORKERS

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NIHL during the first 5 years of noise exposure The NIHL after more than 5 years of noise exposure is of the same magnitude as the hearing loss resulting from 10–20 years of age The NIHL in older age groups was of a minor degree Permanent use of hearing protectors was associated with good hearing but the data suggest that good hearing might be the cause and not the effect of the use of hearing protection

As part of the noise abatement scheme in the Carlsberg and Tuborg Breweries in Copenhagen, pure tone audiometry has been performed since 1973

MATERIAL AND METHOD

During the first year, 6904 workers were examined, which is close to 100% of all employees A thorough medical history with regard to ear- and hearing disorders was taken by audiometrists

Pure tone octave audiometry covering the frequency range 250–8000 Hz, and also including 6000 Hz, was carried out in soundproof booths with a sound level not exceeding 35 dB (A), by means of Madsen TAN 60 audiometers calibrated in accordance with ISO R389 TDH 39 earphones in circumaural muffs ME70 were used Bone conduction and Weber tests were used to appropriately determine the hearing losses as being either conductive or perceptive In order to avoid a significant TTS, all tests were performed at the very start of a working day

A diagnosis system fitted for EDP processing was developed (Fig 1 in which also the frequency 3000 Hz is shown) For each person a two-digit number indicates the diagnosis When the average threshold for 500 1000, and 2000 Hz (TC) is less than 22 5 dB the diagnosis is 1, 2, 3, or 4 depending on the worst threshold within the high frequency range When the TC is more than 22 5 dB and the audiogram curve slopes towards the high frequencies, and the hearing loss is of perceptive type, the diagnosis is 5, 6, or 7 according to the TC An audiogram that does not meet these requirements is grouped under "other hearing loss" and the diagnosis is 8

In this presentation, four main groups of diagnoses are shown

1) Normal hearing in both ears
2) High frequency hearing loss (HFHL) in at least one ear (with a TC less than 22 5 dB)
3) Hardness of hearing (HOH) in at least one ear (TC more than 22 5 dB, sloping towards high frequencies, perceptive loss)

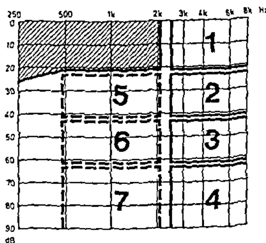


Fig 1 Diagnosis system

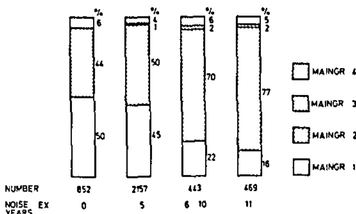


Fig 2 Distribution in diagnosis main groups Age <40 years

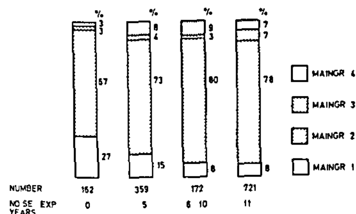


Fig 3 Distribution in diagnosis main groups Age 40–49 years

4) Other hearing losses (one ear may be normal)

A person belongs to the groups 2 or 3 if just one of the ears belongs to these groups, and the grouping is dependent on the worst ear with one of these diagnoses as the investigation was designed to find all possible noise induced hearing losses

Table I *Relation between diagnosis and subjective hardness of hearing*

Diagnosis	Total number	Subjective hardness of hearing	
		Number	%
11	1 792	81	4 5
22	1 077	110	10 7
33	386	62	16 1
44	266	74	27 8
55	134	71	53 0

RESULTS

To elucidate the validity of the diagnosis system, each person was asked whether he/she felt any hardness of hearing when away from work

Table I shows that 4 5% of persons with pure tone thresholds not exceeding 20 dB (diagnosis 11) felt hardness of hearing The symmetrical high tone losses, 22, 33, and 44, were associated with increasing percentage of hardness of hearing 53% of persons with TC from 25 dB to 40 dB were hard of hearing, subjectively

The distribution in the diagnosis main groups showed that persons with ear- or hearing disorders in their past history had somewhat worse hearing than those without, but the effect of noise was the same in both groups The following results therefore include all persons tested

Only 50% of persons under 40 years of age (Fig 2) and without industrial noise exposure had normal hearing Exposure to industrial noise for less than 5 years results in significantly more HFHL and HOH ($p<0\ 005$, χ-square test) The increase in HFHL and HOH is unmistakable in the next two noise groups HOH is an infrequent finding in this age group In the age group 40–49 years (Fig 3) the group of persons without noise exposure is very similar in distribution to the 6–10 year group of noise exposure amongst the younger group (Fig 1) In the 40–49 year group HOH does not differ significantly in the different noise groups, but HFHL+HOH is significantly more frequent in each noise group compared with the non noise group

In the age groups 50–59 years and 60–69 years, there were especially many persons with more than 10 years of noise exposure No significant alterations due to noise could be found HOH was found in 10% and 28%, respectively, normal hearing in 3% and 1% respectively

The permanent use of hearing protectors is clearly correlated to good hearing but hearing protectors are especially used by young persons and in particular by those having worked only short time in noise Thus it might well be that good hearing (and the consequent greater annoyance) is the cause of hearing protection, rather than its effect Probably the correlation must be seen in the light of both protection and annoyance

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COMPARISON BETWEEN STATIONARY AND PERSONAL NOISE DOSE MEASURING SYSTEMS

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Abstract In most of the investigations which have been made to survey occupational noise a stationary sound level meter has been used either alone or in combination with a stationary dosimeter In this investigation which was performed in a ship-building yard an ear borne noise dosimeter has been used The L_{eq} values which were obtained have been compared with those of the pocket borne dosimeters In many processes considerable differences exist between pocket borne and ear borne dosimeters

The usual way to estimate the amount of noise in a workshop has been to use an ordinary sound level meter to measure noise at different locations (Hinchcliffe & Harrison, 1976) As sound fluctuates, it is however difficult to estimate the average meter deflection If the fluctuations are slow, a recorder connected to the sound level meter could solve this problem With this system it is of course not possible to register fast peaks which have a duration of only some milliseconds (Hemingway & Christensen, 1973) During the last few years, noise dosimeters have been developed, which are capable of measuring both slow fluctuations and fast peaks These dosimeters measure the total energy which reaches the microphone during the measuring period—normally a workingday The drawback of all these systems is that they are stationary Many investigations have shown that the position of the microphone is of great importance (Brammer & Piercy, 1977, Martin, 1970) The differences can be very large especially if the noise contains impulse noise With a personal dosimeter it is possible to place the microphone close to the entrance of the ear canal though normally it is not possible to register impulse noise in a satisfactory way (Svensson, 1978)

As our aim is to estimate how impulse noise gives rise to hearing impairment, when compared with continuous and slowly fluctuating noise, we have made this investigation in order to show that large differences can be detected, depending on the way in which the measurements have been made

METHODS

This work is part of an investigation in which we are trying to estimate how the hearing impairment is correlated to the energy content of the impulse sound which reaches the ear The investigation has taken place at Kockums Shipyard, Malmö, Sweden In our investigation we have used three different types of sound level dosimeter, B&K 4423, B&K 4424 and Crafon SLD The stationary dosimeter B&K 4423 has been used together with a B&K 2209 sound level meter equipped with a ½ inch B&K 4165 microphone and set at such a range that no overload time was registered The B&K 4424 is a pocket size dosimeter equipped with a ½ inch microphone The Crafon SLD is an ear borne sound level duration meter which utilizes photographic film as the storage element It measures the time during which the sound pressure has exceeded two preset levels During the individual measuring, the Crafon SLD is borne one on each ear From the exposure times it is possible to calculate the L_{eq} value with an error of ±2 dB (Erlandsson

Table I. *Comparison between different noise dosimeters*

Test run	L_{eq} value dB (A) Crafon 85–130 5 dB steps	B&K 4424	B&K 4423	Crafon 85–115 10 dB steps
1	89 9	90 4	91 5	89 4
2	91 1	88 0	88 7	90 4
3	87 9	88 5	89 9	88 5
4	88 7	88 8	91 1	88 8
5	87 9	89 1	90 9	88 0

et al , 1976) The calibration of the equipment was performed with a pistonphone B&K 4220 and a sound level calibrator B&K 4230

As the behaviour of the personal dosimeters varies, when exposed to impulse noise (Svensson, 1978), the response of the dosimeters was checked with the sound from a mechanical hammer (Erlandsson et al , 1978)

PERFORMANCE AND RESULTS

Usually two Crafon SLDs are borne, one on each ear The normal preset levels are 85, 95, 105 and 115 dB (A) To get an estimation of how much the accuracy of the calculated value is increased when several levels are ',' a special test series with five Crafon SLDs having preset levels of 85, 90, 95, 100, 105, 110, 115, 120, 125 and 130 dB (A) was performed In this series, the Crafon SLDs were placed on a stand, together with the microphones of a B&K 4424 and a B&K 4423 The microphones were grouped close together to be in the same sound field The results are presented in Table I They are in very good agreement The differences between the L_{eq} values calculated with the 5 dB steps (column 2) and with the 10 dB steps (column 5) are very small This supports the assumption that four preset levels are enough for calculating the L_{eq} value in this sound environment In Fig 1 the results from 50 measurements with ear-borne and breast-pocket borne dosimeters have been plotted This investigation was performed on 10 subjects with different occupations The L_{eq} values from the pocket borne B&K 4424 were compared with L_{eq} values calculated from two ear-borne Crafon SLD dosimeters with preset levels of 85, 95, 105 and 115 dB (A)

We found considerable discrepancies between the results from the pocket borne dosimeter and the results from the ear borne dosimeters when we tried to measure the noise from carbon arc gouging This is a process where a light-arc from a carbon electrode together with compressed air, cleans the slag

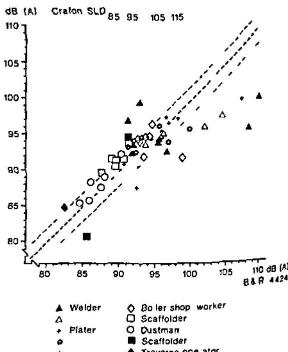


Fig 1 Comparison between L_{eq} values of pocket borne and ear borne dosimeters

Table II L_{eq} *values obtained at carbon arc gouging*

Dosimeter	Microphone location	L_{eq} value dB (A)
Crafon SLD 90/100 110/120	Ear canal entrance	107 8
B&K 4424	Helmet	113 4
B&K 4424	Breast pocket	117 5
Stationary dosimeter B&K 2209+4423	30–80 cm from the ear at the level of shoulder	111 0

from welding grooves To explain these deviations we performed a more detailed investigation for this special case A microphone was mounted on a helmet close to the ear The signal from the microphone was fed to a B&K 4424 In order to measure the noise dose in the vicinity of the ear the microphone of a B&K 2209 was held 30–80 cm from the ear at shoulder level and the signal was then fed to a B&K 4423 stationary dosimeter Comparisons were made between the doses measured by the stationary system, the pocket borne, the ear-borne and the helmet borne systems The results are shown in Table II and Fig 2

DISCUSSION AND CONCLUSIONS

To be able to explain the relation between hearing impairment and the noise dose to which the subject has been exposed, it is important to know the hearing threshold and the noise dose with the greatest possible accuracy

To achieve sufficient accuracy when measuring the noise dose, we had to measure at the very entrance of the ear canal with a system which had the capacity of measuring even impact noise of very short duration

In the hammer test under laboratory conditions (Erlandsson et al , 1978) the L_{eq} values calculated from the waveform patterns agree very well with the noise dose from the large B&K 4423 dosimeter The L_{eq} values calculated from Crafon SLDs are also in good agreement with the B&K 4423 in the hammer test and in the test at the shipyard

Although large deviations may occur between the L_{eq} values from the B&K 4424 and the B&K 4423 in the hammer test, the shipyeard test showed that both the Crafon SLD and the B&K 4424 have an accuracy which renders them suitable in the comparison of the L_{eq} values measured at the pocket and the ear positions For most of the work processes, the Crafon SLD gives an L_{eq} value which is about 2 dB higher than the value measured at the breast pocket position (Fig 1) This may be due to the repetition rate of the impact sounds (Erlandsson et al , 1978) It is often a specialized type of work that gives the big differences

A closer look at the case of the worker who was working with carbon arc gouging (Table II, Fig 2), shows that the L_{eq} value at the pocket position is 4 1 dB higher than that at the helmet position, which in turn is 5 6 dB higher than the Crafon value measured at the entrance to the ear canal The large B&K 4423 dosimeter with the microphone at the shoulder position gave a value 3 2 dB higher than the

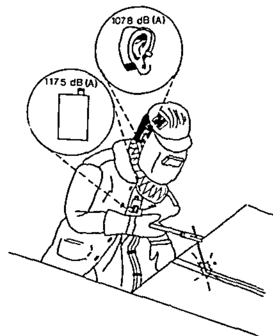
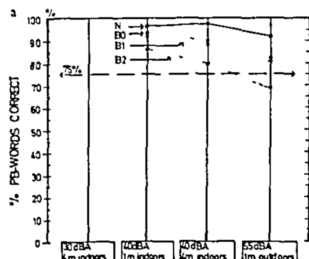


Fig 2 L_{eq} values measured with dosimeters at different positions on a worker performing carbon arc gouging



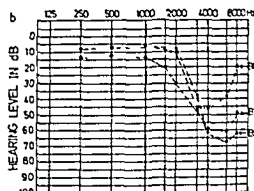


Fig 2 (*a b*) Noise induced hearing loss Men Age 18–50 (N=Normals) Speech intelligibility in random traffic noise, indoors and outdoors Speech level held constant in all tests (70 dB at 1 m) The average pure tone audiograms (better ear) of the groups are also given

PB words (phonetically balanced monosyllables), presented via a loudspeaker (with a directivity pattern and spectrum in agreement with that of an authentic male voice) were recorded over a listening head at distances of 1 m and 4 m indoors and 1 m outdoors (anechoic chamber), using a method described by Aniansson (1974) The speech and noise were presented to the listeners via binaural headphones A total of nine word lists (50 PB words per list) were used—three in each listening situation The listening situations were randomized

The speech level was held constant in all tests (70 dB at 1 m) To determine 75% intelligibility the noise level was adjusted as follows

Indoors

1 m and 4 m
Reverberation time 0 5 sec
List 1 40 dBA
List 2 Level adjusted re % correct in list 1
List 3 Level adjusted re % correct in lists 1 and 2

Outdoors

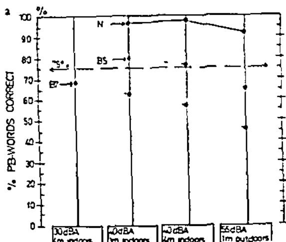
1 m
Reverberation time 0 sec
List 1 55 dBA
List 2 Level adjusted re % correct in list 1
List 3 Level ajusted re % correct in lists 1 and 2

Ordinary unilateral discrimination scores at comfort level were obtained for all hearing impaired subjects The lowest average score for any group was 72% (better ear) Thus there is no significant central hearing loss in any of the test groups

The relationship between the perception of sentences and monosyllables is known from The American National Standard method for the calculation of the articulation index (AI) A 75% intelligibility of PB words corresponds to a 97% understanding of sentences For normals the articulation curve is rather shallow down to 75% intelligibility of PB words below that point the curve falls steeply This means that a noise level which reduces intelligibility to 50% need only change slightly in order to alter perception dramatically Perception scores of 75% correspond to an AI of 0 5 which is the lowest AI for good listening conditions

Figs 1–3 reproduce the speech intelligibility levels in the traffic like noise for some of the most interesting groups, in the three listening situations The average pure tone audiogram (better ear) of the groups is also shown

It is generally agreed that loss of hearing becomes a handicap when an individual can no longer hear speech adequately Currently



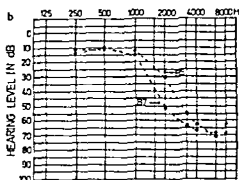


Fig 3 (a b) Noise induced hearing loss Men Age 51–64 (N=Normals) Speech intelligibility in random traffic noise indoors and outdoors Speech level held constant in all tests (70 dB at 1 m) The average pure tone audiograms (better ear) of the groups are also given

however, the most widely used method of estimating the degree of hearing loss for both compensation and prevention purposes is pure tone audiometric testing It is thus very important to know the correlation between pure tone loss and speech discrimination scores in everyday listening The results of this study on noise impaired subjects, especially from the younger groups give us guidance regarding such a correlation It is obvious that as little a loss as 25–35 dB at 2 000 Hz (group B2) results in a significant handicap in noise at 55 dBA The speech interference level of the noise for this group is about 15 dB lower in all listening situations than it is for normals Losses of 40 dB or more at 3 000 Hz and normal hearing at 2 000 Hz (group B0 and B1) give discrimination losses of about 10% and speech interference levels that are 5–10 dB less than in normals About 500 000 persons in Sweden work under very noisy conditions (Statistiska Centralbyrån 1974) For the patients with noise induced hearing loss group B5 represents the common elderly (51–64) man who works or has worked in noise About 50% of all men of that age with an occupational background have hearing corresponding to B5, or worse, according to a running investigation of more than 33 000 workers in industry (Bilsom) This group (B5) cannot tolerate a noise level outdoors of 55 dBA 5–10 dB less would, however, give them 75% intelligibility at 1 m distance

Among the presbycusic groups the P0 and P5 averaged pure tone audiograms agree with the results obtained with those from 70-year-old men and women in Gothenburg, according to an investigation by Bjuro-Moller (1977) This means that about 850 000 of Sweden's 8 million inhabitants have hearing similar to P0 and P5 It is obvious that at least the men of that age have hearing problems outdoors, at 1 m distance, without lip-reading A noise level of 45 dBA in public parks etc would be the highest tolerable level for 70-year-old men, instead of 55 dBA, the present recommended highest level

All clinicians know that speech discrimination ability in persons with conductive hearing loss is not negatively affected by noise One explanation for this was thought to be a rise in the voice level, of the normal hearing speaker, when speaking in noise In this investigation the speech level was constant (70 dB at 1 m) indoors and outdoors Nevertheless the groups with conductive hearing loss all

showed a slight increase in their intelligibility at 55 dBA as compared with 40 dBA This contrasted with all other groups, which showed a decrease It is probable that the difference in reverberation time (0 sec outdoors, 0 5 sec indoors) is the cause of the increase Obviously the condition of the middle ear of these groups protects the almost intact coch leae from the masking noise to a higher degree than is possible in normals and sensorineural losses

In some groups, whose performance indoors at 40 dBA at 4 m distance was rather poor, tests were performed at lower noise levels The gain in speech intelligibility at these lower levels was rather small and the difference only statistically significant (5%) for groups P2 and B7 Thus, the intelligibility gain by lowering the noise level below 40 dBA indoors is rather small

On the other hand a recommended noise level of 55 dBA is too high to allow good speech understanding without lip reading at a distance of 1 m, for groups representing more than 600 000 of Sweden's 8 million inhabitants

Outdoors noise levels in parks etc should not exceed 45 dBA if good listening conditions at 1 m distance, are required Lower noise levels would result in better speech intelligibility which would benefit people with a minimum hearing loss as defined below

1 Most men of 70 or older
   Pure tone audiogram
   1 000 Hz=Normal
   2 000 Hz−25–35 dB
2 A great number of elderly women with presbycusis
   Pure tone audiogram
   1 000 Hz=25–35 dB
   2 000 Hz=25–35 dB
3 About 50% of all men ranging in age range 50–70 who are working or have worked in noisy conditions
   Pure tone audiogram
   1 000 Hz=Normal
   2 000 Hz=25–35 dB
4 All younger men (below 50) with severe noise injuries
   Pure tone audiogram
   1 000 Hz−Normal
   2 000 Hz=25–35 dB

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BINAURAL HEARING SPECTACLES WITH "NO MOULD" BY ACOUSTIC TRAUMA

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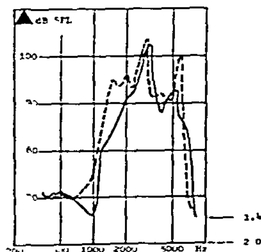
Abstract Patients with selective hearing loss in the trebles as commonly seen in acoustic trauma often have a considerable hearing handicap They seldom benefit from the usual hearing aids with closed ear moulds When Ewertsen (1970) and Courtois (1972) reported their experiences with binaural fitting of hearing spectacles using a free plastic tube in the ear canal (no mould) we at the Hearing Centre in Namsos began to fit such hearing aids to 33 patients with acoustic traumas All of them were followed-up in February–March 1978 The results have been evaluated on the basis of answers from the patients entered on questionnaires and speech audiometry in open field monosyllable with 60 dB wide band background noise 27 patients (80%) stated that they were greatly satisfied and 7 (20%) well satisfied The average daily use was 4½ hrs All of the patients emphasized the great benefit obtained with the hearing aid at conferences meetings social occasions etc The average hearing gain was 13 dB Among those who had discrimination loss there was an average gain of 30%

The Hearing Centre at Namdal Hospital serves a population of about 120 000 people in Central Norway The main source of income is agriculture, forestry, mining industry and construction work Acoustic trauma in these occupations has increased during the years after the last world war as a result of increased mechanism and efficiency Since the middle sixties the situation has improved owing to the cooperation of the Hearing Centre, the company health services and the state labour commission

Most patients with acoustic traumas have selective hearing loss in the trebles from 1 000 Hz They perceive the background noise of everyday life and have usually no problem in tete a tete conversation in quiet surroundings In group conversation, however, or when there also is a background noise, problems arise The fact is that they lose high frequency consonant information and at the same time are disturbed by low frequency background noise Their handicap has a varied degree of importance according to their profession The problem, no doubt, is greater for a person in a leading administrative occupation than for example an industrial labourer or lumberjack All of them have trouble in different social situations being with their family, at parties, political and professional meetings etc People have difficulty in understanding their problem, which often leads to misunderstanding and irritation, and the patients show a tendency to withdraw and isolate themselves Some patients complain of tinnitus which often grows worse by stress and fatigue It is well known that these patients have little if any benefit from usual hearing aids with closed mould

When Ewertsen (1970) and Courtois (1972) reported on encouraging experiences with the binaural fitting of hearing spectacles, using a free plastic tube in the ear canal, the so-called "no mould", among patients with high tone loss, we began to take an interest in this subject, especially with our patients with acoustic trauma in mind In 1973 one of our audiometry assistants went to the Hearing Centre in Århus, Denmark Here he was kindly received and instructed, and from 1974 we also began fitting these devices

As hearing spectacles of this type in Norway cost over 4 000 kroner, it was partly for financial reasons that we selected patients with acoustic trauma in the first place These patients get all their costs covered if the case is


Fig 1

reported and acknowledged as an occupational disease by the national social insurance system Other patients must meet 50–60 % of the expenditures themselves

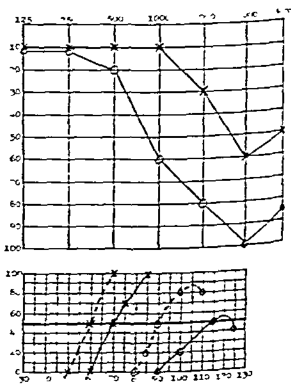
According to Berland and Courtois, patients obtain a gain of 20–25 dB without feed back when using no-mould fittings The gain has a maximum peak at 3000 Hz The frequency response characteristic varies with the inner diameter of the plastic tube and the size of the ear canal, so that plastic tubes with an inner diameter of 2 mm give a greater gain for the medium frequency than a tube with a diameter of 1 2 mm The size of the ear canal has a reverse significance

To begin with we had four tube diameters on trial, namely 1 2, 1 4, 1 8 and 2 mm After a comparatively short while, however, we settled for only two calibres, namely 1 4 and 2 mm We have not taken any special consideration to the size of the ear canal, as, unfortunately we had no technical possibility of measuring amplification and frequency response characteristic in the ear canal of our patients

Fig 1 shows a frequency response curve measured in the ear canal with tubes of 1 4 and 2 mm bore according to a test made by Berland and Courtois in 1972

Our material consists of 33 patients who have been fitted with [illegible]ural hearing spectacles with "no mould" during the 3 year period 1974–77, including 3 women and 30 men The age range was 20 to 80 years average [illegible] years, and the trial period from 3 to 4 years average 14 years 6 patients had previously tried out ordinary hearing aids with closed mould, unsuccessfully All the patients have been examined at our Hearing Centre during February–March 1978 The results have been evaluated firstly from the patients answering questionnaires, and secondly from speech audiometry in open field, without and with hearing aid The tests were carried out with monosyllable word lists, with 60 dB wide band background noise

26 patient (80 %) stated that they were greatly satisfied with the hearing spectacles 7 patients well satisfied All of the patients used their hearing spectacles daily 8 patients used the aid all day, these had administrative occupations in business, industry, banking, etc. The remaining 25 % used the aid only on social occasions, outside of working hours Average daily use was estimated as 4½ hrs The patients


Fig 2

who had troublesome tinnitus reported that this was subdued when using the aid 3 patients expressed joy in hearing the chirping of birds A 45 year old managing director emphasized that he no longer felt so tired and stressed at work as before None reported having technical problems with their aids

In the above mentioned speech audiometry in free sound field with hearing spectacles 15 patients (45%) showed a discrimination of 100% The remaining 18 patients (55%) had a loss of discrimination from 10% to 60% The same test with the hearing spectacles gave an average gain of 13 dB in the hearing threshold (50% comprehensibility) Among those who had discrimination loss there was an average gain of 30%

Fig 2 shows pure tone and speech audiograms of 2 patients respectively the best and the poorest hearing curves in our material The dotted line in the speech audiogram shows the gain of hearing in these 2 patients

The result of this follow up examination is so encouraging that we have the impression that these patients are among our most grateful hearing aid users The reason why is most likely due to the three circumstances pointed out by Dr Ewertsen in 1970 Firstly they obtain a selective amplification in the treble, where they have their hearing loss Secondly, they have a normal supply of low tone and medium frequency tone, and thirdly, the benefit of stereophonic sound impression

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THE NOISE PROTECTION EFFECT OF THE STAPEDIUS REFLEX

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Abstract The purpose was to estimate the attenuation and protection effect of the acoustic stapedius reflex on the inner ear The acoustic stapedius reflex was recorded by means of extratympanic manometry (ETM) This method was used, as it is able to differ between the stapedius and the tensor reflex Patients with unilateral Bell s palsy were investigated during palsy time and after recovery The amplitude of the contralateral acoustic stapedius re [illegible] temporary threshold shift was shown on the affected side (average 34 dB) The hearing threshold on the healthy side was unchanged Thus the attenuation effect of the acoustic stapedius reflex protects the inner ear from an acoustic damage A protection effect of the tensor tympani was not demonstrated

During the last century several theories have been proposed concerning the function of the middle ear muscles Animal, human and model experiments have shown, however, that the protection theory is the most realistic one

Extratympanic manometry (ETM) has been used to demonstrate the attenuation effect of the middle ear muscles—and thus the protection effect of the inner ear ETM is the measurement of air pressure changes in the sealed external ear canal ETM provides a simple way of using homo- contra- and bilateral sound stimulation of any sort, including white noise ETM gives information about the direction of eardrum movements The method is able to distinguish between the stapedius and the tensor contraction contrary to the acoustic impedance method The acoustically evoked tensor contraction was seen as a component of the generalized startle reaction and in that way it was not regarded as a reflex In anima experiments, however, contraction of bot muscles was the "usual" reaction to acoust stimuli

Subjects were investigated during and afte recovering from a unilateral facial paralysi (Bell's palsy) to estimate the attenuation effec of the acoustic stapedius reflex The amplitud of the contralateral acoustic stapedius reflex at stimulus' cessation, was used as a relativ measure of the excitation of the cochlea on th affected side The stimulus difference in dl between "before recovery" response and "af ter recovery" response, corresponding t equal amplitude, was suggested to be a meas ure of the attenuation effect The maximum in dividual attenuation ranged from 15 to 33 dB The attenuation effect was approximately 1 dB measured 20 dB above the reflex threshold i e only 5 dB were transmitted to the cochle by a 20 dB stimulus increase over the reflex threshold

The hearing threshold was tested before and after the ETM investigation After the ETM investigation the ear on the Bell s palsy side showed a maximum TTS from 20 to 55 dB (average 34 dB) The hearing threshold of the healthy ear was unchanged, although this ear had received more burst stimuli After re covery the ear on the earlier affected side ex hibited normal hearing and no TTS after a cor responding ETM investigation One of the most interesting things was, however, that the stapedius reflex had a protective effect 3 oc taves above the stimulus frequency (see Figs 1 and 2)

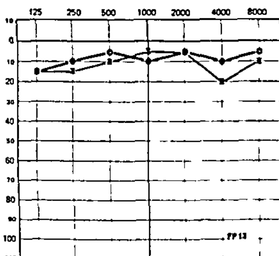


Fig 1 Audiogram from a patient (FP 13) during a right sided Bell's palsy before the ETM investigation

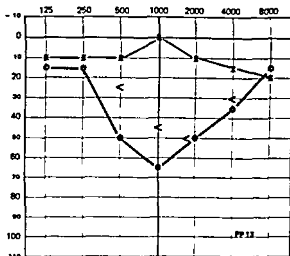


Fig 2 Audiogram from a patient (FP 13) during a right sided Bell's palsy after the ETM investigation (stimulus 500 Hz) The largest TTS was 55 dB

It can be emphasized that the protection effect is much greater than the one which can be concluded from the attenuation effect When the attenuation effect is able to keep the sound transmission to the cochlea below the damage level, the sound protection of the ear is then complete When this damage level is reached, there is no simple correlation between the attenuation effect and the damage which will then depend on the sound parameters Furthermore, it can be concluded, in this study, that the cochlea damage was not dominated by transient sounds The healthy ear, without TTS, was thus unprotected from the tone burst stimulus during the latency time of the stapedius reflex, when the stapedius reflex gave no protection No noise protection could be demonstrated in the tensor tympani recordings

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THE EFFECT OF THE STAPEDIUS REFLEX ON ATTENUATION AND POSTSTIMULATORY AUDITORY FATIGUE AT DIFFERENT FREQUENCIES

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Abstract The effect of the stapedius reflex on attenua

lateral stapedius muscle function The attenuation for a 0 5 kHz tone was found to begin at the reflex threshold to increase about 7 dB per a 10 dB increase in the stimulus tone and to reach a maximum in the vicinity of 20 dB When a 2 0 kHz tone was used no attenuation of note appeared until the sound had risen to about 10 dB above reflex threshold The attenuation reached a maximum at a little less than 10 dB A properly functioning stapedius muscle significantly reduced poststimulatory auditory fatigue at 0 75 kHz while at 3 0 kHz it could do so only if it was stimulated into action by low frequency noise

The ear is very susceptible to noise induced damage within the frequency range 4–6 kHz Within lower frequency ranges, the susceptibility is considerably less The possible explanations for this are many One factor which might be of importance is the attenuating capacity of the stapedius reflex Fig 1 (from Borg & Zakrisson 1974) shows the attenuation brought about by the reflex as a function of stimulus intensity at 0 5 kHz The attenuation is already in action at the reflex threshold, increases about 7 dB per a 10 dB increase in the stimulus and reaches a maximum in the vicinity of 20 dB Brask (1977) found an attenuation effect of 15 dB at 20 dB above the reflex threshold In a study on patients with unilateral facial palsy and stapedius muscle paralysis, Zakrisson (1975) showed that the temporary threshold shift (TTS) at 0 75 kHz reached significantly higher values in the ear with the stapedius muscle paralysis at intensities from 110 dB SPL and above after the patient had been exposed to narrow band noise with a center frequency of 0 5 kHz (see Fig 2, showing the TTS mean values in the affected ear and in the normal ear upon exposure to noise of various intensities) Brask (1977) found that patients with facial palsy had a maximum TTS of 55 dB at 1 kHz in the affected ear and no TTS at all in the normal ear after exposure to the sound eliciting the stapedius reflex

It is generally believed that the stapedius reflex does not attenuate sounds with frequencies from about 1 5 kHz and upward The purpose of the study to be presented here was to find out whether the stapedius reflex attenuates sound at 2 kHz and to observe the reflex's effect on TTS after exposure to a continuous pure tone at 2 kHz

MATERIAL AND METHODS

The study was carried out on 22 patients with unilateral facial palsy (Bell s palsy) and stapedius muscle paralysis (Experiment group I) and on 6 normal test subjects, with a normal bilateral stapedius muscle function (Experiment group II) The stapedius reflex was measured quantitatively during both contralateral and ipsilateral stimulation according to the impedance method introduced by Møller (1961) All of the patients in Experiment group I suffered a total loss of the contralateral stapedius reflex, while some of them had a weak ipsilateral reflex with a high threshold All of the persons in both of the experiment groups

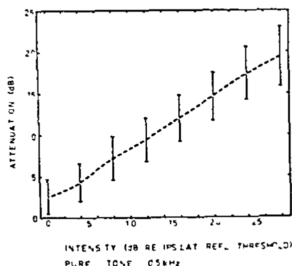


Fig 1 Interrupted line shows Mean values ± Standard Deviation (vertical bars) of the attenuation provided by the stapedius reflex as measured in 19 Bell's palsy subjects (from Borg, Zakrisson, 1974)

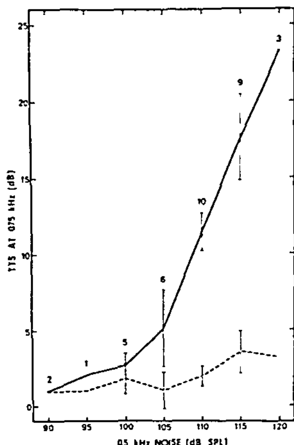
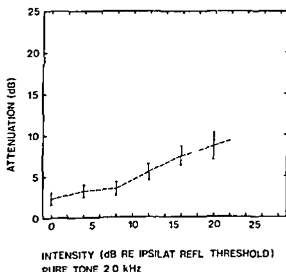


Fig 2 Mean values ± Standard Errors of the Mean (vertical bars) for TTS during paralysis as a function of the intensity of the exposure noise — affected ears ---, normal ears Exposure duration 5 min The number of values at each separate intensity is indicated Eighteen Bell's palsy subjects with unilateral stapedius muscle paralysis (from Zakrisson, 1975)

had normal eardrums and normal hearing up to and including 4 kHz The vestibular function, examined by means of calorization and electronystagmography, showed nothing of note Fourteen of the patients in Experiment group I regained a normal stapedius muscle function after a few months The attenuation at 2 kHz in these patients was measured by comparing the contralateral reflex responses on that occasion (after recovery) with the corresponding values found during acute paralysis

All of the subjects in Experiment group II and 13 of those in Experiment group I (during acute paralysis) were exposed to a 2 kHz continuous pure tone with an intensity of 110 or 115 dB SPL The tone was presented to each ear for 2 min The hearing threshold before and after the exposure was registered with a pulsating Bekesy tone set at 3 kHz TTS was measured 2 min after the end of the exposure After several days, when the hearing threshold had returned to normal, the TTS measurements were repeated on the respective ears The same exposure conditions held as earlier On the latter occasion, the patients in Experiment group I were exposed simultaneously in the contralateral ear to bandpass filtered steady-state noise with a center frequency of 0 5 kHz and a bandwidth of 0 3 kHz at 3 dB points (Brüel & Kjær, type 1024, filter). The intensity of the noise was adjusted so as to give a maximal reflex amplitude in the normal ear The contralateral noise did not give rise to any reflex at all in the ear with the stapedius muscle paralysis. This was a given condition, since only patients with a total loss of the contralateral stapedius reflex had been selected for the group In Experiment group II, the noise was presented contralaterally to 3 persons and ipsilaterally to the other 3 The



group I

noise was adjusted from one recording to another so as to give between 35–100% of the maximal reflex amplitude obtained

RESULTS

Fig 3 shows the attenuation curve at 2 kHz The entire curve is flatter than the same type of curve plotted for the 0 5 kHz tone (see Fig 1) When the frequency was 2 kHz, no attenuation of note appeared until the sound had risen near to 10 dB above reflex threshold The attenuation 20 dB above the threshold was about 8 dB The corresponding value for the 0 5 kHz tone was 14 dB

Fig 4 shows the mean TTS values at 3 kHz for the affected ear and the non-affected ear in the patients in Experiment group I after exposure to a 2 kHz pure tone with and without contralateral noise The presence of the contralateral noise with an optimal reflex activity gave rise to a significant reduction in TTS in the normal ear In the affected ear, no such effect was observed

When 5 of the patients in Experiment group I were exposed to an ipsilateral 2 kHz pure

TTS MEAN (dB)

	Affected ear	Nonaffected ear
Ipsilateral 2.0 kHz pure tone	16 6	20 2
Ipsilateral 2.0 kHz pure tone Contralateral 0.5 kHz noise	14 8	9 4
Difference	1.8	10.8
Significance	-	p < 0 001

Fig 4 TTS mean values at 3 kHz after exposure to a 2 kHz pure tone in the presence and in the absence of 0 5 kHz noise in the affected and the normal ear Thirteen subjects of Experiment group I

tone only, the TTS had its average maximum at 2 6 kHz in both the affected ear and the normal ear It would therefore seem that the non-significant difference between the normal ear and the affected ear upon exposure to only a 2 kHz pure tone is probably not ascribable to different TTS-maxima with versus without a functioning stapedius reflex Zakrisson (1975) found the same degree of difference in TTS in patients with facial paralysis after exposing them to narrow-band noise at 2 kHz

In Experiment group II, the contra or ipsilateral noise had to be such as to induce a reflex activity of at least 80% in order to reduce the TTS at 3 kHz when the ear was exposed to a pure tone of 2 kHz The results varied from individual to individual as well as between the two ears of one and the same individual

DISCUSSION

The study shows that, contrary to previous opinions, the stapedius reflex attenuates sound at 2 kHz and, under certain circumstances, causes the TTS to undergo a significant reduction after exposure to sound at 2 kHz The reflex can thereby play a protective role for the cochlea in this frequency range as well This may be one reason for the general observation that the susceptibility for a permanent threshold shift (PTS) is pronounced first at frequencies of 4 kHz and above

Reflex decay at 2 kHz was a consistent finding in both experiment groups This decay most likely explains why exposure to only an ipsilateral 2 kHz pure tone in the normal ear gave rise to a significantly greater TTS than the TTS arising when the reflex was activated optimally during the entire exposure time In order for the stapedius reflex to have an attenuating effect at 2 kHz, it must be activated to reach a great amplitude and it must be brought into action by a sound within a frequency range where the endurance is high (Andersson et al , 1969) The narrow band noise with a center frequency at 0 5 kHz used in the experimental study presented here fulfilled those criteria and can be termed ' protective noise or ' protective sound

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NASOCYTOLOGIC EXAMINATION OF WOOD INDUSTRY WORKERS

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Abstract To establish a methodology for field examination for early diagnosis of ethmoidal cancer in workers in the furniture industry two methods were compared namely nasal rinsing and direct sample taking from the middle meatus *The latter method proved superior* resulting in a high number of cylinder cells This method was tried out in a field survey of 715 furniture workers No tumours or precancerous changes were revealed The investigation continues by exposing the Syrian golden hamster to wood-dust and using amongst other methods quantitative nuclear morphometry for evaluation

Epidemiologic investigations in Britain, France, Belgium, Denmark and Sweden have demonstrated that there is a clear over representation of cancer in the ethmoidal region in wood workers (Acheson et al , 1967, Hadfield & Macbeth, 1969, Andersen et al , 1977, et al , 1977) The disease is rare, even woodworkers, but a study by Ache et al (1967) reported a thousand fold risk increase compared with other occupational categories The latent period for the development of ethmoidal cancer ranges from 27 to 45 years, whilst the minimum exposure period appears to be around 8 years Even though this has not been definitely established, it appears that *wood dust as such is the only common* denominator, whilst solvents and the like have not been proved to constitute a risk factor It seems, in addition that the timber species is of a certain importance For instance no increased risk was demonstrated for individuals who had worked exclusively with spruce and pine

From the point of view of industrial protection, it must be desirable that there should be a method for the early detection of ethmoidal cancer in a relatively high risk group such as woodworkers, and also if possible to identi the precancerous condition

We have therefore undertaken such a stud both with the intention of evaluating vario sample taking methods suitable for the dete tion of cell changes in the nasal mucous mem brane in this field and of laying down certa *guiding principles for an animal experiment* study in order to examine the natural histor and possible etiology of ethmoidal cancer i the Syrian golden hamster

MATERIAL AND METHODS

In a preliminary study, we compared a metho in which we rinsed the nose with a nasal de congestant (Otrivin 0 1%, Ciba) with a metho in which we took direct samples from the mid dle meatus by means of a probe In both cases the material was fixed in a mixture of methan and acetic acid and a differential calculatio of the cell composition was performed in th 17 cases where both sampling methods wer used

The direct sampling method was subse quently used in a health investigation surve undertaken on 715 wood workers from an are in Sweden in which there is a concentratio of timber firms

Sample taking methodology

Direct sampling proved superior to the rinsin method, as the samples obtained by the forme contained up to 88% cylinder cells as com pared with only 2% when rinsing with a de

congestant As actual cancer cases are adenocarcinoma a method resulting in a maximum of cylinder cells is to be preferred

In a small number (2%) of the wood workers examined some metaplastic changes were observed as were certain discrete nuclear changes not evident at renewed controls This implies that no cancerous or precancerous changes could be identified in any of the 715 wood workers examined up to the present time

DISCUSSION

The principal aim of the survey presented was to establish a good sample taking methodology for the early diagnosis of ethmoidal cancer As no tumour could be demonstrated in the series it is difficult to judge whether either of the methods used meets the demands to be made on a methodology for the early diagnosis of cancer As 88% of the direct sample cells were cylinder cells it seems obvious that the direct sample is preferable to the rinse sample Apart from the cell composition the sample taking method performed under visual control during rhinoscopy is of course better than the rinse method

Considering the frequency of ethmoidal cancer (4–5 patients per annum in the Swedish furniture industry which has 17000 employees) it is statistically not unreasonable that no cancerous or precancerous case was detected in the 715 persons examined We have judged it necessary further to elucidate the natural history of ethmoidal cancer by the study of tumour development in an experimental system using the Syrian golden hamster exposed to wood-dust perhaps in combination with nitrosamines The changes that occur in the mucous membrane of the experimental animal will be traced using a newly developed technique for quantitative nuclear morphometry (Stenkvist et al , 1978) Thus, it will be possible to characterize the normal mucous membrane in objective terms and eventually to establish the process leading to the development of ethmoidal cancer in the Syrian golden hamster It may then be possible to reveal analogies with the natural history of human ethmoidal cancers

Although up to now the results of attempts to diagnose ethmoidal cancer at an early stage in woodworkers have proved negative, it is of great psychological importance to those individuals engaged in the furniture industry to know that efforts are in fact being made for the early diagnosis of ethmoidal cancer, which they known can threaten their lives of some of them

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OCCUPATIONAL ETIOLOGY AND NASAL CANCER

An Internordic Project

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Abstract Reports from Britain and other countries have shown an increased frequency of adenocarcinoma of the nose and paranasal sinuses among workers exposed to fine wood dust With support from the Nordic Council a joint project has been launched in Denmark Finland Norway and Sweden to single out occupations and exposures possibly associated with nasal cancer The project is a prospective case referent study with matched control cases of colon cancer Each new case of malignant epithelial tumour of the nose and sinuses reported to the individual national cancer registries is interviewed concerning previous occupational exposures according to the program The estimated duration of data collection is two years This type of joint study is new to the Nordic countries and we hope to gain valuable experience in order to improve cooperation within this type of medical research A retrospective study in Sweden has shown that 50% of all male cases of nasal adenocarcinoma from 1960 to 1972 were joiners most of them cabinet makers The duration of exposure to fine wood dust was known in 21 of 22 cases The latency period varied from 22 to 70 ars with a mean value of 44 7 years The exposure varied from 9 to more than 30 years The results correspond to those reported from Britain and Denmark

An increased frequency of adenocarcinoma of the nose and paranasal sinuses among cabinet makers was reported by Acheson et al (1967) They found that workers exposed to fine wood dust were particularly at risk Reports from France (Gignoux & Bernard, 1969, Gignoux et al , 1971), Belgium (Debois, 1969), Holland (Delemarre, 1971), Australia (Ironside & Matthews, 1975), Denmark (Mosbech & Acheson, 1971, Andersen, 1975) and Sweden (Engzell et al , 1978) have confirmed these findings An unexpected high incidence rate of carcinoma of the nose and sinuses has also been reported among workers in the leather and textile industries (Acheson et al , 1970 Achesen et al , 1972) and among workers exposed to nick (Doll et al , 1970)

A prerequisite for the preventing of occupa tional carcinoma is a knowledge of the causa relationship Therefore, it would appear justi fied to explore the risk of contracting nasa cancer in the Nordic countries With suppor from the Nordic Council we have started joint project in Denmark, Finland, Norwa and Sweden intended to single out occupation and exposures possibly associated with nasa cancer

In each one of the four countries a projec group is at work on a national basis, accord ing to a joint program Each new case of malig nant epithelial tumour of the nose and sinuse reported to the individual national cancer reg istries is reached by contacting the reporting doctor A national interviewer will then collec data from each subject concerning previou occupational exposure according to the join program The project is a prospective case referent study and the controls will be matched cases of colon cancer The estimatec duration of data collection is 2 years

This type of joint study is new to the Nor dic countries and we hope to obtain valuable experience from this project in order to im prove Nordic cooperation within this type of medical research

We are at present occupied in surveying pre vious occupations and exposures of a series of cases of nasal cancer in Sweden A prelimi nary report will appear shortly in *Acta Oto-laryngologica* All cases in Sweden from 1960

Table I *Occupation of males with adenocarcinoma, 1961–72*

Occupation	No	Percentage
Joiners	22	50
Building workers	1	2
Fitters	9	20
Transport workers	3	7
Flour workers	3	7
Miscellaneous	6	14
Total	44	100

to 1972 have been traced with the assistance of the National Cancer Registry and the Swedish Occupational Environment Fund By interviewing those subjects still alive and by collecting information from relatives and the population registry we have been able to compile relevant data concerning the duration of exposure among joiners and carpenters who have been suffering from adenocarcinoma of the nose

In a series of 44 male cases of adenocarcinoma 50% were joiners, most of them cabinet makers Table I shows the distribution of the occupations The number of subjects working in the flour industry was also higher than expected Fig 1 illustrates the length of occupational exposure to fine wood dust in 21 of 22 cases The latency period, i e the time from the beginning of the exposure until the time of diagnosis, varied from 22 to 70 years (mean

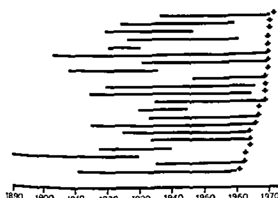


Fig 1 Duration of exposure to wood dust and diagnosis in 21 joiners

Table II *Occupation of males with squamous cell and poorly differentiated carcinoma, 1965–70*

Occupation	No	Percentage
Joiners	5	4
Other woodworkers	8	6
Building workers	10	8
Fitters	23	18
Farm labourers	23	18
Stone drillers	3	2
Transport workers	6	5
Textile workers	6	5
Flour workers	1	1
Leather workers	1	1
Miscellaneous	25	20
Unknown	16	13
Total	127	101

value 44 7 years) The exposure period was in general more than 30 years but only 9 years in one subject who had been working exclusively in an oak parquet floor factory Incidentally we happen to have a corresponding case on our ward just now—a previous oak parquet floor worker with only 8 years of similar exposure

As a comparison, Table II shows the occupational distribution among men with squamous cell and poorly differentiated carcinoma of the nose This is a series based on a 6-year material, compared with the previous one based on 12 years The latter series has not yet been duly scrutinized

Finally I would like to draw the attention to the vast blank spaces within the research concerning the relation of the environment to diseases within our specialty, not only tumours

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KARTAGENER'S SYNDROME—A REAPPRAISAL

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Abstract Congenital lack of dynein arms in cilia has recently been associated with Kartagener's syndrome Ultrastructural findings from six patients with chronic stagnant secretion in the respiratory tract are briefly reported Only one of the patients had the complete syndrome The term Immotile-cilia syndrome has been coined and it is suggested that Kartagener's syndrome should be included in this entity

Following the description by Kartagener (1933) of four patients with complete transposition of the viscera, bronchiectasis, and chronic hypertrophic rhinosinusitis with nasal polyposis, this clinical entity has been called Kartagener's syndrome or triad Subsequent observations of oligosymptomatic forms and of the occurrence of severe and disabeling disease in the siblings of patients with the classical triad, have led to uncertainty as to whether Kartagener's syndrome merits recognition as a separate clinical entity

Torgersen (1952) suggested that males with Kartagener's syndrome probably were infertile this additional feature of the syndrome being subsequently confirmed by Arge (1960) in a detailed family study Recent investigations of male infertility have revealed a group of patients with live but immotile spermatozoa (Afzelius et al, 1975, Pedersen & Rebbe, 1975) Some of the latter were previously recognized cases of Kartagener's syndrome, whilst others suffered from chronic respiratory disease, and at the same time had siblings with the complete classical triad Semen from these patients demonstrated lack of dynein arms bridging between the nine outer double filaments in the sperm tails Dynein is an ATPase responsible for ciliary motility (Satir, 1974) Against this background it seemed conceivable that lack of dynein arms in cilia might be associated with Kartagener's syndrome

MATERIAL AND RESULTS

In a sibship with eight members, one male, aged 28 years, had Kartagener's syndrome diagnosed in 1972 A brother aged 23 and a sister aged 21 both had chronic rhinosinusitis with nasal polyposis and chronic bronchitis The remaining five siblings did not have increased frequency of respiratory infections Transmission electronmicroscopic investigation of cilia from respiratory epithelium in these patients demonstrated lack of dynein arms They therefore suffer from an identical enzymatic defect which produces varying clinical manifestations

Persons exhibiting neither *situs inversus* nor bronchiectasis may therefore have ciliary immotility An 11-year-old boy who has been under constant treatment during the last 6 years for chronic secretory otitis media and recurrent nasal polyposis, was exmined with respect to the presence of dynein Two of his three siblings also had hypertrophic rhinosinusitis with polyposis and moderate though persisting symptoms of secretory otitis media Absence of dynein in nasal mucosal biopsies was confirmed electronmicroscopically in all three siblings Bronchiectasis has not been demonstrated in any of the latter patients, but all of them have a productive cough caused by chronic bronchitis

The normal mucous transport by the respiratory epithelium depends on motile cilia The goblet cells and seromucinous glands, however, are well developed in the present

condition, leading to enormous amounts of stagnant secretion throughout the respiratory tract This lifelong bronchitis may eventually lead to bronchiectasis

Further electronmicroscopic investigations have revealed secondary changes of the respiratory mucosa of varying severity Irregular internal organization of the structural elements of the axoneme is common as well as vesicle formation of the ciliary membrane and formation of megacilia where many axonemes are found within the same ciliary membrane Surface areas devoid of cilia, but with microvilli still present, are common An intra-epithelial oedema leads to desquamation of the superficial layer of the respiratory epithelium and subsequent denudation of the basal cell layer White blood cells are frequently found in the epithelium and in the overlying secretion Different stages of secondary changes may be found in adjacent regions in the same patient Biopsies from the maxillary sinus have shown areas with destruction of the basal membrane and extensive desquamation of cell debris and collagen fibrils intermingled with epithelial remnants

CONCLUSIONS

Kartagener's syndrome may now be considered part of a disease state caused by lack of the ATPase dynein in cilia, resulting in clinical symptoms from the respiratory tract The eponym should no longer be used, and this inborn enzymatic defect should rather be named the Immotile cilia syndrome, as suggested by Eliasson et al (1977) thus including the various clinical manifestations of the condition Patients with persisting and therapy resistant rhinosinusitis and bronchitis from early infancy should be investigated with the possibility of congenital lack of dynein in mind

ZUSAMMENFASSUNG

sind kurz beschrieben worden Nur einer der Patienten hatte das komplette Syndrom Der Begriff unbewegliches Ziliensyndrom ist vorgeschlagen worden und weiterhin wird vorgeschlagen dass auch das Kartagener Syndrom unter diesem Begriff geführt werden soll

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MUCUS PRODUCTION IN THE NASAL SINUSES

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Abstract. The density of goblet cells and glands was determined in the maxillary, ethmoidal, frontal and sphenoidal sinuses. Goblet cells showed the highest density in the maxillary sinuses, 9700 per mm^2, while in the other sinuses their density was approximately the same, from 5900 to 6500 cells per mm^2. The density of glands was highest in the ethmoids, 0.5 gland/mm^2, then in the maxillary sinuses, viz. 0.2 gland/mm^2, while in the frontal and sphenoidal sinuses it was very low, 0.08 and 0.06 gland/mm^2 respectively.

Very little is understood about the production of mucus in normal nasal sinuses, but the transport function by the respiratory epithelium of the sinuses is known to be fairly effective (Messerklinger, 1967). In the present paper we wish to compare the mucus production in the various sinuses mutually and in relation to the nose. This can be done by comparing the density of mucus producting elements—mucous glands and goblet cells—in the various sinuses. In this way it is possible to determine the ability of the mucous membrane to produce mucus. Apart from the quantitative studies of mucous elements carried out in our Laboratory, the literature contains no data concerning the density, number, and distribution of the mucous elements.

MATERIAL AND METHODS

The material comprises mucous membranes from 10 maxillary sinuses removed post mortem from 10 patients by the Luc Caldwell approach, from 32 (16 right and 16 left) frontal sinuses and 32 ethmoidal sinuses removed from another 16 patients by way of the anterior cranial fossa, and from 32 sphenoidal sinuses removed through the sella turcica and clivus. All the patients had died of malignant or cardiovascular disease, and none had shown signs of acute or chronic diseases of the nose or nasal sinuses.

Most of the mucosa was removed and stained by the PAS–alcian blue whole mount method and divided as follows. Maxillary antra, frontal and sphenoidal sinuses into a superior, inferior, anterior, posterior, medial and lateral wall. The ethmoid was divided into an anterior and medial part—belonging to the anterior cells and posterior part with posterior cells.

Goblet cells were counted in each wall in up to ten 0.01768 mm^2 fields and glands in 3–20 four mm^2 areas. The mean density in each wall was calculated. Details of the method and detailed results for the individual sinuses have been published already (Mogensen & Tos, 1977*a*, 1978; Tos et al., 1978*a*, *b*), so that only differences (if any) between the sinuses will be analysed here.

RESULTS

In all sinuses the epithelium was pseudostratified, columnar, ciliated, and 25–50 μm thick. The basement membrane was fairly thin. So was the lamina propria, which contained a few round cells and some fibrocytes. At depth, collagen fibrils passed, without sharp limits, to the periosteum. Mucosal thickness was 0.2–0.7 mm in the frontal and sphenoidal sinuses, and 0.3–0.8 mm in the maxillary antra and ethmoids, thickest close to the ostia and in the wall in which the ostium was situated.

Goblet cell density. None of the sinuses showed statistically significant differences in interindividual median density between the

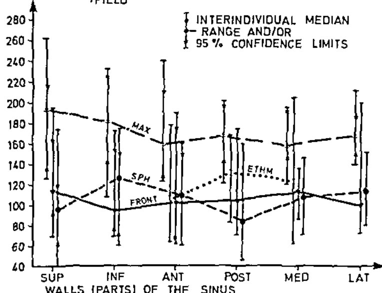


Fig 1 Interindividual median density of goblet cells in different walls of the different paranasal sinuses

various walls or parts (Fig 1) (Mann Whitney test, $p>0.05$) Thus, the density in the walls close to the orifice was the same as in those far from it However, the range was fairly wide, though equally wide in all sinuses

The individual median density, based upon all counts from a patient, from the right as well as left sinus, exhibited a relatively wide range (Table I) However, the interindividual median density was almost the same in the frontal, sphenoidal, and ethmoidal sinuses, corresponding to 5 900–6 500 cells per mm² The goblet-cell density was highest in the maxillary sinus (Fig 1) 9 700 cells/mm², in which it was significantly higher ($p<0.01$) than in the ethmoidal sinus which was the next highest (Table I)

Table I *Interindividual median density of goblet cells and range in the paranasal sinuses*

Sinus	Cells per 0 01768 mm² field		Cells/mm² median
	Median	Range	
Maxillary	172	122–204	9 700
Ethmoidal	115	63–194	6 500
Frontal	104	69–176	5 900
Sphenoidal	109	36–140	6 200

Glands

In the lamina propria there were tubulo alveolar, seromucous, round to oval glands the majority having an area of 0 3–0 5 mm² and being 100 μm thick Except for a small area of 20–30 mm² around the ostia, where the glands were arranged in an approximately continuous, thin layer, there was no glandular layer in the greater part of the sinusal mucosae, the few glands being scattered singly in the mucosa which had large areas entirely devoid of glands

In the maxillary sinuses the glands were found in all 57 walls studied, in the ethmoid in all 88 parts studied In the sphenoidal sinus no glands were found in 25 % of 183 walls studied and in the frontal sinus in 37 % of 166 walls studied Thus, in the frontal sinus there were significantly fewer walls with glands (χ^2 test $p<0.02$) than in the sphenoidal sinus

The interindividual density of glands in the *individual walls* (Fig 2) was significantly higher in the frontal sinuses in the juxta-orificial, inferior part (Mann Whitney test, $p<0.008$) than in the others In the maxillary sinuses too the density was significantly higher in the juxta orificial medial part ($p<0.003$) In the sphenoidal sinuses there was also a tendency to a higher density in the anterior wall

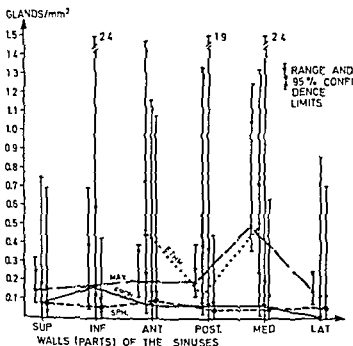


Fig. 2. Interindividual density of glands in different walls of the different paranasal sinuses.

in which the density was significantly higher ($p<0.008$) than in the posterior wall, where it was lowest. A relatively high density was observed also in the superior wall which is in close relation to the orifice which is found superiorly in the anterior wall. In the ethmoids there was a significantly higher ($p<0.001$) density in the anterior and medial parts—both of which are from the anterior ethmoidal cells —than in the posterior cells.

The interindividual median density for the *entire sinus* was lowest in the sphenoidal and frontal sinuses (Table II), but the differences between these two sinuses were not statistically significant ($p>0.05$). The density was highest in the ethmoids, next highest in the maxillary antra, with a statistically significant difference ($p<0.01$) between the two. The difference in median density between the maxillary antra and frontal sinuses was also significant ($p<0.006$).

DISCUSSION AND CONCLUSION

The density of goblet cells id believed to be highest in areas with little air current (Hilding, 1932). The high density in the lateral wall of the inferior turbinate (Mogensen & Tos, 1977*b*) and in the maxillary antra might support this theory, but the appreciably lower density in other sinuses, in which there is also no air current, indicates that each region of

Table II. *Interindividual median density, range, and 95% confidence limits of glands in paranasal sinuses*

	No. of sinuses	Density glands/mm²		
		Median	Range	95% confidence limits
Maxillary	10	0.20	0.09–0.32	0.09–0.32
Ethmoidal	32	0.47	0.07–0.89	0.26–0.61
Frontal	30	0.08	0–0.57	0–0.13
Sphenoidal	32	0.06	0–0.43	0.05–0.1

respiratory epithelium has its habitual goblet cell density However, it cannot be ruled out that the higher density in the maxillary antra as compared with the other sinuses is due to previous pathological actions which have subsided The patient material from which the maxillary antra were obtained was not the same as that from which the other sinuses were removed, but it seems reasonable to assume that the frequency of affections in the maxillary is probably no higher than in the ethmoids

There was a significantly higher density of glands in the walls in which the ostium is situated than in the other walls This difference is due to embryological factors and the postnatal growth of the sinuses The density of glands was highest in the ethmoids, thereafter in the maxillary antra, frontal and sphenoidal sinuses Thus quantitative histology is able to disclose the different capacity of the various nasal sinuses to produce mucus As a whole, mucus production by the glands is very slight in relation to that by the goblet cells We have previously demonstrated that in most sinuses there are only a few glands—fewer than 50 in the frontal and sphenoidal sinuses and 50–100 in the ethmoids (Tos et al, 1978*a* *b*, Mogensen & Tos, 1978) As the glands are not big their total mucus producing capacity is very small in relation to that of the goblet cells which thus produce the greater part of the mucus in the nasal sinuses In this repsect the mucous membrane of the nasal sinuses differs essentially from that of the nose which has a gland density of 7–10 glands/mm² and about 36000 glands on the septum plus the middle and inferior turbinate (Tos & Mogensen, 1977) From a physiological point of view this difference is of importance showing that the mucus production in the nasal sinuses is accurately adapted to their requirements As there is no air current in the nasal sinuses, there is little need for protection of the mucous membrane by mucus This protection is adequately taken care of by the goblet cell mucus which is involved in the mucociliary clearance

ZUSAMMENFASSUNG

Die Dichte der Becherzellen und Drusen wurde in der Kieferhohle Stirnhohle Siebbeinhohle und Keilbeinhöhle bestimmt Becherzellendichte war am hochsten 9700 Zellen per mm² in der Kieferhohle in den anderen Nebenhohlen war sie einigermaßen gleich 5900–6500 Zellen per mm² Die Dichte der Drusen war am hochsten in der Siebbeinhohle 0 5 Drusen per mm² danach in der Kieferhohle mit 0 2 Drusen per mm² wahrend die Dichte in der Stirnhohle und der Keilbeinhohle sehr klein war 0 08 beziehungsweise 0 05 Drusen per mm²

RESUME

Tæthed af bægerceller og glandler blev bestemt i sinus maxillaris ethmoidalis frontalis og sphenoidalis Bægercelletæthed fandtes hojest i sinus maxillaris 9700 celler per mm² men's den i andre kæbehuler er nogenlunde ens 5900–6500 celler per mm² Tæthed of glandler var hojest i sinus ethmoidalis 0 5 gld /mm² dernæst i sinus maxillaris 0 2 gld /mm², mens den i sinus frontalis og sphenoidalis var meget lav henholdsvis 0 08 og 0 06 gld /mm²

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A POPULATION STUDY OF OTITIS MEDIA IN CHILDHOOD

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Abstract A total of 494 children with an average age of 3½ years were studied with a view to elucidating the frequency of both acute and secretory otitis media and their relationship if any to a number of social factors Of the children 41% had had at least one attack of otitis media in 49% of these associated with aural discharge 70% of the children were attending day-care centres and the incidence of otitis media was highest in this group According to Jerger's classification of the tympanometric curves 51% of these were of type A 17% of type B and 32% of type C Significantly higher frequencies of types B and C curves were found among children attending day-care centres

The acute and the secretory otitis media are common diseases of childhood

With a view to the elucidation of the frequency of these diseases and their relationship, if any, to a number of social factors a total of 494 children from the municipality of Aarhus, all born in 1974, i e of an average age of 3½ years, were studied

METHOD

During the year 1974 a total of 3500 children were born in the municipality of Aarhus The children studied were divided into four groups as shown in Table I, 33 children were included in two, or sometimes three, groups, thus giving a total number of cases of 527

Of the series of 494 children studied, 236 were girls and 258 boys The children accompanied by one of the parents were summoned by letter for examination, 86% of those concerned appeared for examination In all cases, a medical history was taken but in 4% physical examination and tympanometry were not performed because the children were unwilling to submit to the procedures

The clinical study consisted in the taking of a thorough somatic and social history with answers to a total of 93 standardized questions, including many subquestions, an otological examination and tympanometry

As the study was retrospective, the diagnosis of acute otitis media was based on the information given to the parents by the doctors who had treated the children

RESULTS AND DISCUSSION

In the four groups, 26% of the randomly selected children had previously had otitis media, as against 42% living in newer houses in a residential suburb, 41% living in poor housing conditions and 63% of those previously admitted to our ENT department In all, 41% had had otitis media at least once, and these children had, on the average, had 3 8 attacks If the most seriously affected group, viz those who has previously been admitted to our department, is excluded, an average of 36% in the first three groups had previously had acute otitis media Of all the children with previous otitis media, 49% had had aural discharge, and there was no significant difference among the four groups The first attack of otitis media had occurred before the age of 1 year in 51%, and before the age of 2 in 75% of the children, which was found to be of great importance for the recurrence rate (Fig 1), which showed a linear fall with increasing age This observation is in agreement with those made by Howie (1975), for example, who also reported that children in whom otitis media developes before the age of 1 year carry a significantly

Table I *The number of children studied divided into four groups*

	No
Randomly selected	153
From a newer residential suburb	170
Living under poor housing conditions	142
Previously admitted to ENT department	112
Total	577

Table II *Tympanometric findings (number of ears) related to day care at home or outside home*

Taympanometric curve	At home		Outside home	
	n	%	*n*	%
Type A	180	64	264	45
Type B	26	9	117	20
Type C	76	27	201	35

greater risk of recurrence than those who have their first attack of otitis media after that age

Strangert (1976) showed that in children under the age of 18 months significantly more attacks of otitis media occurred among those who attended day-care centres than in those who were cared for in their homes, whereas no such difference was demonstrated in children over 18 months

In our series, 37% of the children cared for at home had had otitis media as against 45% of the children in day-care centres (day nurseries, babyminders, kindergartens) However, this difference is not significant (p= 0 16), even in children attending day-care centres as early as from the age of 3–6 months

Among other observations, it may be mentioned that there were significantly more attacks of otitis media in children living in flats than in those living in houses The recurrence rate was also higher among children with a birth weight either below 2500 or above 4000 g

On the other hand, we found no correlation between the number of attacks of otitis media and such factors as the social status of the parents, number of siblings, length at birth smoking habits of the parents, or breast feeding, even when this was related to its duration

Tympanometry revealed that according to Jerger's classification, the curves obtained in 51% of the children were of type A, i e normal or slightly negative middle ear pressure, in 17% of type B, i e flat tympanometric curves and in 32% of type C, i e a negative air pressure greater than −100 mmH_2O As regards the 17% with flat curves it is known with great certainty that they have effusion in the middle ear This result is in agreement with those of several previous investigations in which 10–20% showed flat curves

An analysis of the tympanometric curves in the four groups did not disclose any difference, even in the most seriously affected group, i e those who had previously been admitted to our ENT department The tympanometric findings in children who had previously had otitis media did not differ from those in children who had never had otitis media Nor was any correlation revealed between previous adenotomy and the tympanometric findings at the time of examination

The relationship between home care and day-care centres as compared with the tym

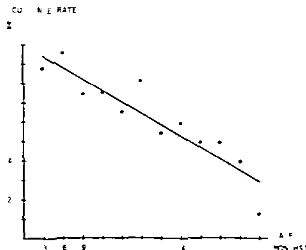


Fig 1 Recurrence rate related to first attack of otitis media

panometric findings is seen from Table II. The preponderance of type A curves in children cared for at home and of B and C curves in children attending day-care centres is significant ($p<0.001$). This difference may be a consequence of a higher frequency of upper respiratory infections in children in day-care centres, which presumably also explains why significantly more children ($p<0.001$) in day-care centres had undergone adenotomy (a total of 101 of the children).

An interesting observation as regards children with bilateral flat curves, i.e. with effusion in the middle ear, was that 73% of their parents had not noticed impaired hearing in their children although an average hearing loss of 25–30 dB was disclosed in them.

As in acute otitis media, no correlation was demonstrated between the tympanometric findings and the following factors: breast feeding, social status, number of siblings, and smoking habits of the parents.

The study reported here, which represents a small part of 1500 EDP tables from the series, calls attention to a few variables which seem to be of importance in the development of acute and secretory otitis media. One of the most outstanding predisposing factors seems to be day-care outside the home. A number of other factors can be elucidated only by comprehensive prospective studies.

ZUSAMMENFASSUNG

Insgesamt 494 K[illegible]der im Alter von [illegible]

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sollte 41% der Kinder hatten mindestens einmal [illegible] Mittelohrentzündung gehabt und davon hatten [illegible] Ohrenfluss gehabt. 70% der Kinder wurden [illegible] ausserhalb des eigenen Heims betreut und das Vorkommen von Otitis media war am höchsten in [illegible]

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fikant höher unter denjenigen Kindern, [illegible] nicht im eigenen Heim beaufsichtigt wurden.

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INFLAMMATORY MIDDLE EAR DISEASES IN PATIENTS WITH CYSTIC FIBROSIS

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Abstract 111 patients with cystic fibrosis (CF) aged 7 months to 29 years were examined to detect inflammatory middle ear diseases 35% had a history of one or more attacks of acute otitis media an incidence at the same level as in non-CF patients None had chronic suppurative otitis Only 4 out of the 88 patients able to cooperate in audiometry had a hearing impairment In 2 the hearing loss was perceptive in 3 very mild and in the fourth case moderately severe The middle ear pressure was measured in 108 patients in 86 of whom it proved normal In 15 patients the middle ear pressure was lower than −100 mmH O indicating tubal occlusion and in 7 patients the impedance minimum was lacking indicating an accumulation of mucus These 7 patients (6%) had secretory otitis media Nasal polyps were or had been present in 32% There was no correlation between nasal polyps and tubal occlusion or secretory otitis The incidence of inflammatory middle ear diseases in CF patients was in the same range as in non-CF patients a finding at variance with some previous investigations showing middle ear pathology in 25–48%

Cystic fibrosis (CF) is a congenital disease affecting the exocrine glands, mucus- as well as non mucus secreting It is an autosomal recessive trait whose aetiology is still unknown The hypothesis has been advanced that an important link in its pathogenesis is altered fluid and electrolyte transport through cell membranes resulting in a reduced fluid-electrolyte fraction in various gland secretions which thereby become concentrated (Johansen, Anderson & Hadorn 1968) This phenomenon is particularly marked in the pancreas and in the mucous glands where the altered, viscid secretion obstructs the excretory ducts of the glands, entailing secondary dilatation of the ducts and acini as well as gradual destruction of the secretory epithelium

Inflammatory middle ear diseases in patients with CF have rarely been studied Kulczycki et al (1970) found conductive hearing loss in 27% of 70 CF patients, including 10% with secretory otitis media Forcucci & Stark (1972) demonstrated middle ear diseases in 48% of 31 patients with CF, secretory otitis media in 42% Fritze et al (1973) reported hearing impairment in 32% of 34 CF patients In contradistinction, Siegel & Taylor (1970) found normal hearing in 23 CF patients investigated over a period of 5 months, and Jerger & Neely (1971) demonstrated hearing impairment in only 8% of 51 CF patients which does not differ from the incidence of hearing impairment among school children as a whole Lastly, Taylor et al (1974) found mild transient hearing impairment in only 10% of 50 CF patients in connection with tubal dysfunction None of these latter patients had suppurative or secretory otitis media

The considerable divergences in previous results as well as the extension of knowledge concerning the mucous elements of the middle ear mucosa during the past decade (Bak Pedersen, 1977) formed the basis of the present prospective study

MATERIAL AND METHOD

During the period April–June 1976 a total of 111 patients with CF were examined when appearing for their appointed monthly follow up in the Paediatric Out patient Clinic They comprised 88% of the CF patients registered at the Paediatric Department of Rigshospitalet, Copenhagen, who were alive at the end

of the study period The remaining patients had a mild form of CF and were not required to attend for follow-up during this period Of the patients, 62 were boys and 49 girls, median age 9 years (range 7 months to 29 years)

A careful otological history was obtained, with particular emphasis on previous acute or chronic otitis media, and a thorough objective otological examination was performed, especially an accurate assessment of the appearance and mobility of the ear drum An impedance test was carried out, using the Madsen impedance bridge, type ZO 70 A lack of impedance minimum in connection with reduced or abolished mobility of the drum in Siegle's otoscope was taken to represent secretory otitis media An impedance minimum at −100 mmH_2O or lower was interpreted as denoting tubal dysfunction The examination also included tone audiometry using Madsen's audiometer, type OB 70, to determine the air and bone conduction threshold If the threshold in the frequency range 125–8000 Hz was 20 dB HL or higher for two or more frequencies, the hearing was designated abnormal

RESULTS

65% had never had suppurative otitis media The remaining 35% had a history of one or more attacks, but only 3% had had more than 3 acute attacks of otitis media No patient had acute or chronic otitis media at the time of examination Clinical signs of sinusitis had been or were present in all cases Thirty patients (27%) had undergone adenotomy and 4 of them also tonsillectomy

Audiometry was practicable in 88 patients (79%), 84 of whom had normal hearing Four had hearing impairment, mild conductive in 2 and perceptive in 2, one of whom had a mild, bilateral high tone loss and the other one severe, unilateral hearing loss for all frequencies In 20 or the 23 patients in whom audiometry could not be carried out tympanometry was done Ten patients had normal middle ear pressure and stapedial reflex thresholds, so that presumably they also had normal hearing Among the other 10, four had flat tympanometric curves and six negative middle ear pressure

Tympanometry was performed in 108 patients (97%) In 86 it was normal, 15 had negative middle ear pressure, and 7 had flat tympanometric curves Among the 86 patients with normal tympanometry, 27% had a history of one or more attacks of acute otitis media, while among the 22 patients with abnormal tympanometry 68% had a history of suppurative otitis media This difference is statistically significant ($p<0.001$) and emphasizes the relationship between acute suppurative otitis media, tubal dysfunction, and secretory otitis media

Nasal polyps had been or were still present in 32% Comparison of the occurrence of nasal polyps with the tympanometric findings showed nasal polyps in 19% of the patients with normal and in 18% of those with abnormal tympanometry This indicates that the presence of nasal polyps apparently does not influence tubal function

Seven patients (6%) had secretory otitis media, bilateral in 4, unilateral in 3 Another 6 patients had exhibited secretory otitis at previous examinations, but not at the present one

DISCUSSION AND CONCLUSION

The very high incidence of rhinopharyngitis and sinusitis among patients with cystic fibrosis, causing almost constant accumulation of muco-purulent secretion around the pharyngeal tubal orifices, combined with sneezing and coughing during infections in other parts of the respiratory tract, might be presumed to cause an appreciably increased incidence of suppurative as well as of secretory otitis media According to the present study, however, CF patients do not have a higher incidence of hearing impairment, of acute or chronic suppurative or of secretory otitis media than non-

CF patients Thus, our findings do not confirm previous reports of middle ear pathology in 25–48 % of CF patients

The season during which the examinations was performed does not appear to have influenced the results Neely et al (1972), examining 93 CF patients in December–January, found an even lower incidence of hearing impairment, purulent and secretory otitis than we did in April–June

CF patients have been found to have characteristic, degenerative changes of the mucous glands in the bronchi, oral cavity and nasal sinuses (Pennington, 1956, Tygstrup et al, 1972) Our own studies, not yet completed, on middle ear mucosa from CF patients indicate that neither mucous glands nor goblet cells differ quantitatively or qualitatively from those in non-CF patients It may seem surprising that the mucous elements of the middle ear mucosa in CF patients apparently escape the degenerative changes which are so widespread in other parts of their respiratory tract Part of the explanation is perhaps afforded by the result of the present clinical examinations, viz that the middle ears of CF patients are not exposed to the same severe inflammatory ac ı as other parts of their respiratory tract

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MIDDLE EAR MECHANICS AND EUSTACHIAN TUBE FUNCTION IN TYMPANOPLASTY

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Abstract In 100 patients with chronic otitis media tubal function and volume was tested before operation In a postoperative study on hearing and healing there was no positive correlation between these findings and the pre operative results In 50 patients with healed ear drums tubal function was retested in a pressure chamber with a flow volume technique An improvement in Eustachian tube function could be demonstrated These findings in dicate that poor tubal function in chronic otitis media might be secondary to other factors responsible for the disease

The debate concerning the role of the Eustachian tube function in middle ear surgery has been going on for many years Several investigations have been described but the results differ widely Miller & Bilodeau (1967), Holmquist (1968) and Siedentop et al (1972) found better healing results in patients with good preoperative tubal function, as compared with those with impaired function In investigations by Ekvall (1970), Lee & Schuknecht (1971) and Virtanen (1977) no such difference was found

Using the test methods and equipment developed by the middle ear research group in Malmo the present study was carried out with the following aims

1 To find out if the tubal function of the volume of the cell system affects healing after surgery for chronic otitis media
2 To find out if there is any change in tubal function when tested postoperatively

METHODS

For preoperative tests the equipment used was that described by Andreasson & Ivarsson (1976) (Fig 1) It consists of an airtight glass syringe with a micrometer screw, hermetically connected via a three way stopcock to a pressure transducer and a digital instrument The ear and the measuring device form a closed system whose pressure is variable The syringe volume is shut off from the rest of the system during measurement of the pressure, making the total volume of the apparatus less than 1 ml By changing the pressure in the system and instructing the patient to swallow, one can manometrically measure tubal function according to the aspiration–deflation method first described by Flisberg et al (1963) With this equipment, Politzer's test, Valsalva's maneouvre and Toynbee's test could also be performed and determination of the volume of the air filled earspaces could be made

For the postoperative analysis the pressure chamber apparatus first described by Elner et al (1971*a*) was used The patient sits in a pressure chamber in which the pressure can be adjusted to over- or underpressure, and the measuring device consists of two identical flow meter systems (Fig 2) One, "the ear flow meter system", is hermetically connected to the outer ear canal Movement of the eardrum by changing the middle ear pressure (ΔP_m) produces a flow in the external ear canal and in the flow meter ($\dot{V}_{tm}$) By integrating the flow signal it is possible to record the volume displacement of the tympanic membrane (V_{tm}) This procedure is used for Valsalva's, Toynbee's and Politzer's tests It is also possible to record the volume displacement of the tympanic membrane when the chamber pressure is

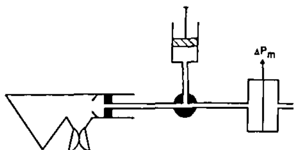
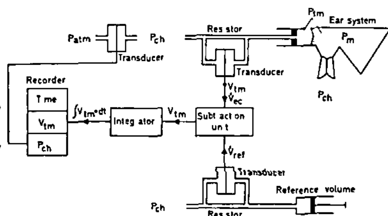


Fig 1 Block diagram of equipment for preoperative testing

changed (ΔP_{ch}) by using an adjustable reference system operating a separate flow meter —"the reference flow meter system When the system is in proper balance, the airflow in the reference system (V_{ref}) and that in the ear system (V_{ec}) neutralize each other Active equilibration of static over and underpressure in the middle ear was studied by the decrease/increase in chamber pressure at ± 10 cmH_2O

During each examination the number of deglutitions was limited to 10 The effect of the pressure equilibration was recorded as a change in the position of the ear drum (Fig 2) This apparatus was also used to determine the initial middle ear pressure (Fig 3) The recording is based on the fact that the movement velocity of the drum is maximal in its neutral position By repeatedly changing the chamber pressure from -15 to $+15$ cmH_2O during which time the patient is requested not to swallow, the drum is pushed outwards or inwards by the relative over- and underpressures created in the middle ear In Fig 3 the maximum velocity (V_{max}) is noted at points *a* and *b* At this moment the pressure in the middle ear is the same as that in the chamber This pressure is defined as the initial middle ear pressure (P_{mi}) relative to the pressure outside the chamber



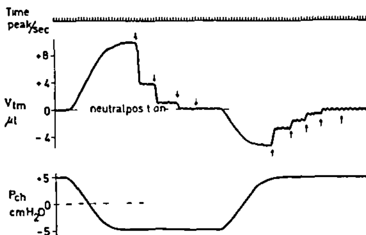


Fig 2 Block diagram of the pressure chamber and flow meter systems

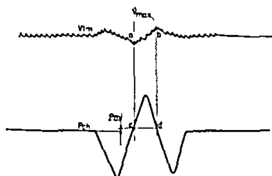


Fig. 3 Calculation of initial middle ear pressure P_{mi}, V_m velocity of the drum P_{ch} chamber pressure For performance see text

For statistical evaluations the χ square test was used

Performance of test

A routine ENT examination was performed and the test ear was examined under an operating microscope Only dry ears with central perforation of the drum were accepted and then only in patients who showed no signs of upper respiratory tract infection when tested or operated upon The tests were done with the patient sitting upright

After placing the polyethylene catheter with cuff in the outer ear canal the system was checked for leakage The tests were performed in the following order

1 Politzer's test
2 Toynbee's test
3 Aspiration–deflation test at ±10 cmH_2O and max 10 deglutitions
4 Valsalva s test
5 Volume determination

Postoperatively the catheter was applied in the same way and the tests were carried out in the following order with the patient sitting upright

1 Initial middle ear pressure P_{mi}
2 Valsalva s test
3 Toynbee s test
4 Aspiration–deflation test at ±10 cmH_2O and max 10 deglutitions
5 Compliance registration
6 In certain cases, ' sniff test

Table I *The age distribution of the material*

Age	No of patients
0–10	3
11–20	8
21–30	26
31–40	21
41–50	22
51–60	14
>60	6
Total	100

Hearing was tested with a pure tone audiometer (Madsen OB 70) together with matched TDH 39 earphones fitted with MX 41 AR cushions for air conduction For bone conduction a bone vibrator from Radio Ear model B-71 was used The audiometric zero for air-threshold was calibrated according to STAF, 1975 Mean air- and bone threshold levels were calculated for the frequencies 500, 1 000 and 2 000 Hz The difference between air and bone conduction is given as the air–bone gap (ABG)

Surgical technique and postoperative care

The operations were performed by four experienced surgeons using a uniform technique Myringoplasty was done with dried temporalis fascia and underlay technique By ossicular reconstruction defects in the ossicular chain were repaired The materials used were autografts of ossicular remnants, ossicular homografts or septal cartilage

Decongestant nosedrops were used for 10 days and phenylpropanolamine for at least 4 weeks or until the middle ear was aerated The patients were instructed to perform Valsalva's manoeuvre when necessary

Table II *Take rate in relation to aspiration capacity and Valsalva s test*

	Pos aspir		Neg aspir	
	n	%	n	%
Healed/asp cap	63/72	88	26/28	93
Healed/Vals test	75/83	90	14/17	82

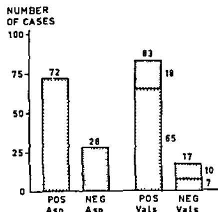


Fig 4 The grouping of the material according to aspiration–deflation test and tubal patency

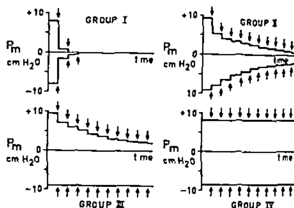


Fig 5 Tubal function groups according to the aspiration–deflation method

MATERIAL

The material consisted of 100 patients (100 ears) with a central perforation following chronic otitis media, and operated on consecutively between 1974 and 1976 There were 47 women and 53 men The age distribution is given in Table I 75 patients underwent simple myringoplasty, the remaining 25, myringoplasty combined with ossiculoplasty The take rate and hearing gain refer to the re-examination one year after the operations The investigation in the pressure chamber was performed between 1 and 3 years after the operation

RESULTS

Preoperatively, Politzer's test was positive in all cases Valsalva's manoeuvre was positive in 83% and Toynbee's test in only 37% The aspiration test was positive in 72% (Fig 4) Use was made of the grouping according to Elner et al (1971*b*) (Fig 5), who divided their material of normal ears into four tubal function groups In our investigation, groups I and II were merged into the positive aspiration group and groups III and IV into the negative The maximum residual pressure tolerated in the positive group was -9 cmH_2O

The total take rate was 89% and there was no difference between cases operated on with myringoplasty, 90% (68/75), or myringoplasty +ossiculoplasty, 84% (21/25) ($p>0.05$) The healing results showed no correlation to the preoperative aspiration capacity or to Valsalva's manoeuvre (Table II) The volume was measured on 86 ears in the preoperative investigation and the mean volume was 3.15 ± 0.28 ml Of these, 76 healed with a preoperative mean volume value of 3.23 ± 0.30 and the remaining 10 with a mean volume of 2.48 ± 0.59

Table III *Percentual distribution of 68 healed myringoplasties in different air–bone gap groups pre- and postoperatively related to the aspiration capacity*

Air–bone gap (dB)	Pos aspir (47) Preop (%)	Pos aspir (47) Postop (%)	Neg aspir (21) Preop (%)	Neg aspir (21) Postop (%)
0–10	6	73	10	76
11–20	32	20	47	19
20–	62	7	43	5

Table IV *Aspiration capacity pre and postoperatively after successful myringoplasty (50 ears)*

Aspiration capacity	Preop		Postop
Positive	34	2	45
Negative	16	13	5

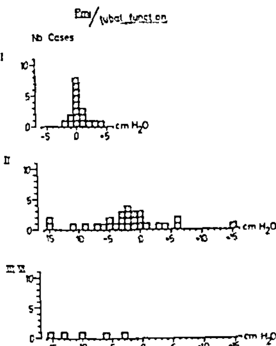


Fig. 6. Initial middle ear pressures (P_{mi}) in relation to tubal function groups.

ml did not heal. There is no statistically significant difference between the mean volume values in the healed and the non healed groups ($p>0.05$).

Since technical failures in the ossiculoplasty group could affect the hearing results, only the hearing gain in the myringoplasty group is presented (Table III). The results are presented as the percentage distribution of patients in different air–bone gap groups: 0–10, 11–20 and 20– dB pre- and postoperatively and related to aspiration capacity. No significant statistical difference in hearing gain between the two tubal function groups was found ($p>0.05$).

Out of the 100 ears in the material, 59 (50 with intact ear drums and 9 with perforations) were retested with the pressure chamber technique. The pre- and postoperative findings are compared in Table IV. Obviously the tubal function improves after successful surgery. Of 34 ears with a positive preoperative aspiration capacity, only two turned negative while 13 out of 16 patients with a preoperative negative capacity turned positive postoperatively.

As for the 9 ears with persistent perforations or reperforations, the figures were somewhat different (Table V). Two ears with preoperative good funftion got worse and lost aspiration capacity. Only one from the negative group showed a positive aspiration capacity. Valsalva's test was positive in 80% (40/50) postoperatively.

Fig. 6 shows the initial middle ear pressures in relation to the tubal function groups according to Elner et al. (1971*c*). In group I with a perfect aspiration capacity the initial middle ear pressures were zero or virtually zero. In group II with slightly impaired aspiration capacity the values varied widely, but with a tendency towards negative middle ear pressures. In groups III and IV without aspiration capacity the initial middle ear pressures were invariably negative. These 5 patients, however, were capable of inflating their ears with the aid of Valsalva's manoeuvre.

The compliance curves showed a variety of findings. Mostly they were very flat, and no definitive patterns in relation to the tubal function could be recognized.

Finally "the sniff test" was performed in 32 cases. Six of those could create an underpressure in the middle ear with this technique.

DISCUSSION

As stated earlier (Andreasson & Ivarsson, 1976) Politzer's and Valsalva's tests are patency tests. Politzer's test was positive in all cases, but this only means that there is no organic stenosis of the Eustachian tube. Ability to perform a positive Valsalva manoeuvre

Table V. *Aspiration capacity pre- and postoperatively in drums with a perforation postoperatively (9 ears)*

Aspiration capacity	Preop		Postop
Positive	7	2	6
Negative	2	1	3

is probably favourable for the postoperative aeration of the middle ear To some degree this depends on training factors As expected, there was no change in the ability to perform Valsalva's manoeuvre in the postoperative investigation Nor is there any correlation between aspiration capacity and tubal patency as tested with Valsalva's manoeuvre the other comprising (Fig 5)

As far as we know the aspiration test best reflects the normal physiological function of the Eustachian tube Two major objections can be raised, however One is that the test period is only 2–3 minutes, which means that we are unaware of any possible fluctuations in the function during a longer period of time The other is that some individuals regularly equilibrate the underpressure created in their middle ears by other ways than swallowing, i e by yawning, by moving their jaws or even by autoinflation The test methods must therefore be further developed and refined

The positive finding in this study is the definitive improvement of the tubal function in healed ears In their investigation Siedentop et al (1968) found a tendency to improvement which they attributed to the surgical procedure, i e removal of granulations and polyps at the tubal orifice of the middle ear In our material, however no such lesions were seen in the 13 ears transferred from the negative to the positive aspiration group (Table IV) The most probable explanation for this is some change in the middle ear mucosa Since the volume of the mucosal lining is highly dependent on the blood filling a change could be explained by the altered environment in the closed middle ear Another explanation might be that in ears with perforated drums there is no pressure difference across the Eustachian tube, but when the ear is closed a more physiological situation occurs with repeatedly created underpressures which might function as a possible trigger mechanism inducing the tube to start functioning

In our study the preoperative volume did not influence the outcome of the operation This is not in accordance with Holmquist's & Bergstrom's (1978) findings In order to obtain a better comparison between the materials we divided ours into two groups, one with a negative aspiration capacity and a volume value below 3 ml (17 ears), and the other comprising the rest of the material in which the volume was measured (69 ears) A volume value of 3 ml corresponds to an area on the X ray of about 9 cm² (Andreasson, 1976) which is a critical value according to Holmquist & Bergstrom The healing results in the first group were 87% (15/17) and in the rest of the material, 87% (60/69) which does not mean any significant difference ($p>0.05$)

According to recent findings by Ekvall & Magnusson (1977) the "sniffing habits of the patient might be an important pathophysiological factor in middle ear disease In our postoperative study, 6 out of 32 (19%) could propagate a negative rhinopharynx pressure to their middle ears We believe that this mechanism might only in some cases contribute to the problems in middle ear pathology

The tubal function tests now in use as well as the volume determination of the air filled ear spaces seem to be of no value for selecting patients suitable for middle ear surgery The postoperative improvement of the tubal function in healed ears indicates that an impaired tubal function is probably secondary to other factors responsible for the development of chronic otitis media We feel that continued research on the pathophysiological mechanisms in chronic otitis media is called for

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MIDDLE EAR PRESSURE FOLLOWING TYMPANOPLASTY FOR VARIOUS MIDDLE EAR DISEASES

Pressure Related to Follow-up Period and Retractions

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Abstract In 512 ears with various middle ear diseases the middle ear pressure was measured a minimum of 2 years and a maximum of 10 years after tympanoplasty The findings were related to the length of the follow up period and to retractions of the drum and/or in the epi tympanum The tympanometric findings were best in sequelae to otitis with dry perforations poorest in cho lesteatomas and adhesive otitis Tubal function had not further deteriorated 4 years after the operation There was a highly significant correlation between the middle ear pressure and the frequency of retractions, which also does not essentially increase—except in the cholesteatoma cases

In a previous study (Tos, 1974) I analysed—on the basis of a material of 534 ears with various diseases, operated upon from 1963–70 and seen at follow up in 1972—the functional results in relation to tubal passage before, 3–9 months after, and 2–10 years after tympano plasty

In the present study I analysed the middle ear pressure *in relation to follow-up period* after the operation in another material subjected to operation during the period 1971–75 and seen at follow-up in 1977 and 1978 It was the intention to ascertain whether the middle ear pressure deteriorates several years after the tympanoplasty and how much correlation there is between the degree of negative pressure and retractions of the drum as well as retraction pockets in the epitympanum To my knowledge, such studies have not been reported in the literature Booth (1973) analysed tympanometry and compliance after successful tympanoplasties in relation to the graft used, but the present study deals with the entire material available from a given period and comprising several disease conditions

MATERIAL AND METHODS

The material, which comprises 512 ears, was divided into group I, viz 250 ears subjected to operation in 1974 and 1975, and group II, con sisting of 239 ears subjected to operation in 1971–73 and 23 operated upon in 1967–70 In late 1977 and early 1978 the patients were seen at follow-up which included tympanometry audiometry, and otomicroscopy Thus the follow-up period for group I was a minimum of 2 years and a maximum of 4 years, that for group II a minimum of 4 and a maximum of 10 years, but in the majority of cases 7 years at most Tympanometry was carried out with the Madsen ZO 70 impedance meter in the fre quency range 220 Hz, and the middle ear pres sure was classified into four groups (Table I) The pre-, intra-, and post-operative investiga tions and treatment of tubal function as well as the tympanometry findings in relation to Valsalva's manoeuvre, tubal pathology, and postoperative middle ear ventilation have been reported previously (Tos, 1978*a*)

RESULTS

Sequelae to otitis These ears were dry at the time of operation and were treated by my ringo or tympano-plasty with ossiculoplasty but without mastoidectomy The postopera tive middle ear pressure in relation to the size and site of the perforation has been described previously (Tos, 1978*a*) There was no differ ence in relation to the size and site of the per foration, but in ears with middle ear tympano-

Table I *Sequelae to otitis Middle ear pressure related to postoperative observation time*
Figures in parentheses are the percentages of ears with retracted drum

Groups	No of ears		Middle ear pressure in mmH_2O A 0 to −99		C_1 −100 to −199		C_2 −200 to −350		B Flat curve		Perforation
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	(%)
Group I	113	(9)	73	(4)	12	(8)	6	(43)	6	(43)	3
Group II	110	(4)	73	(3)	14	(0)	4	(25)	2	(50)	8
Total	223	(6)	73	(3)	13	(4)	5	(36)	4	(44)	5

sclerosis the tympanometric findings were significantly poorer than in others

Among the total of 223 ears with sequelae to otitis (Table I) 73% had type A tympanograms and only 5% recurrence of the perforation There were no statistically significant differences in the distribution of tympanograms between group I and II (χ^2-test $p>0.05$) In group II the percentage of re perforations was somewhat higher than in group I, the reverse was found for the percentage of tympano grams of types C_2 and B

A retracted or adherent drum was found in 6% of all 223 ears (Table I, percentages in parentheses) A retracted drum was present in a significantly higher percentage of ears having type C_2 (36%) and type B (44%) than in those having types A and C_1 ($p<0.001$) The frequency of retraction did not differ significantly between groups I and II

Thus, it may be concluded that the tympanometry findings are fairly favourable in sequelae to otitis, that they do not deteriorate with increasing follow-up period, that a constantly negative pressure is the most common cause of postoperative retraction of the drum, and that the retraction probably does not increase 4 years after tympanoplasty There were no differences in the frequency of retraction between total, anterior, inferior, and posterior perforation or between ears with ossicular defect without perforation

Cholesteatomas In 71 ears there was an attic cholesteatoma with perforation in the pars flaccida, in 67 sinus cholesteatoma with a posterosuperior perforation, and in 46 ears a pars tensa retraction cholesteatoma The tympanometry findings were a little better in those with sinus cholesteatoma than in the other two groups (Tos, 1978*a*) Most cholesteatomas were treated in one stage by modified intact-wall mastoidectomy and tympanoplasty (Tos, 1978*b*), 27 of them by reconstruction of the auditory meatus, obliteration, and tympanoplasty in the same stage

Group II showed somewhat poorer tym-

Table II *Cholesteatomas Middle ear pressure related to postoperative observation time*
Figures in parentheses are the percentages of ears with retracted drum or/and retraction pockets

Groups	No of ears		Middle ear pressure in mmH_2O A 0 to −99		C_1 −100 to −99		C_2 −200 to −350		B Flat curve		Perforation (%)
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	(%)
Group I	97	(35)	22	(5)	18	(18)	30	(54)	22	(60)	8 (14)
Group II	92	(50)	25	(0)	11	(70)	22	(50)	36	(88)	6 (17)
Total	184	(42)	23	(2)	15	(37)	21	(52)	29	(75)	7 (15)

Table III *Chronic adhaesive otitis Middle ear pressure related to postoperative observation time*

Figures in parentheses are the percentages of ears with retracted drum

	No of ears		Middle ear pressure in mmH O									
			A 0 to −99		C_1 −100 to −199		C_2 −200 to −350		B Flat curve		Perforation
Groups	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	*n*	(%)	(%)
Group I	19	(32)	32	(17)	16	(33)	32	(17)	16	(100)	5
Group II	25	(60)	12	(33)	8	(50)	40	(80)	36	(56)	4
Total	44	(48)	21	(22)	11	(40)	36	(56)	27	(67)	5

panometry findings (Table II), there being a larger number of type B than in group I, but conversely more type C_2 than in group I In all, 60% of the ears in group I had type C_2, B, and perforation, compared with 64 in group II, but this difference is not statistically significant ($p>0.05$)

Retractions in the epitympanum or in the tympanic sinus were significantly more common ($p<0.005$) in group II, viz in 50% of all ears as against 35% in group I (Table II) In both groups there was a statistically significant correlation between the tympanometric findings and retractions ($p<0.001$) In group II even 70% of type C_1 ears had retraction More than half the cholesteatomas in the attic had retractions in the epitympanum, viz one third group I ears and over two-thirds of those in group II in which the retractions were also more pronounced Almost one third of the sinus cholesteatomas had retractions, predominantly in the tympanic sinus—in this case too mostly in group II Almost half the pars tensa cholesteatomas had retractions, in some cases also adhesions of the drum, but this was more common in group I

Thus, the investigation of patients with cholesteatomas showed particularly poor tympanometric findings, a high percentage of retractions which were to a marked extent related to the negative pressure and partly also to the length of the follow up period

Chronic adhesive otitis These patients were treated by tympanoplasty possibly ossiculoplasty, as a rule without mastoidectomy The tympanometric findings as a whole were even poorer than in the cholesteatoma cases (Table III), there being only 32% ears with types A and C_1 In group II the findings were poorer than in group I ($p>0.05$)

The percentage of retractions was higher in group II, 60% of all ears, than in group I (32%), though this difference is not statistically significant ($p>0.05$) The frequency of retraction increased with decreasing middle ear pressure, but retractions did occur also in types A and C_1 (Table III)

Chronic granulating otitis This group represents 61 ears with chronic discharge, a granulating mucosa, central perforation of varying size, but without cholesteatoma Despite prolonged conservative treatment before the operation these ears could not be rendered dry They were treated by classical intact wall mastoidectomy and tympanoplasty in the same stage The tympanometric findings were considerably better than in cholesteatomas and in adhesive otitis, almost 48% of these ears being type A 15% type C_1, 15% type C_2 and 10% type B and 13% had a reperforation

There were no significant differences ($p>0.05$) in tympanometric findings or in the frequency of retractions between groups I and II On the whole, the frequency of *retractions* was essentially lower (16%) than in cholesteatomas or adhesive otitis, whereas the frequency of re-perforations was highest in granulating otitis The great majority of perfora

tions were small, situated anteriorly and representing permanent ventilation. Several of them were not detected until tympanometry.

Total material. In all, tubal function was no poorer in group II than in group I. Group I consisted of 250 ears including a total of 20% ears with retractions as against 27% in group II which consisted of 262 ears. This difference is not statistically significant ($p>0.2$). There was a significant correlation between middle ear pressure and retractions. Among 222 ears with type A tympanogram, 10 (40%) had retractions, usually mild. Among 69 ears with type C_1, 14 (20%) had retractions—a significantly ($p<0.001$) higher percentage than in type A. Among 84 ears with type C_2, 43 ears (51%) had retraction, also a highly significant ($p<0.001$) difference from type C_1. Among 80 ears with type B, 54 ears (68%) had retractions, also a significantly higher ($p<0.05$) percentage than in type C_2.

DISCUSSION AND CONCLUSION

Very considerable differences in tympanometric results were found between the various disease states. The poorest findings were in cases of cholesteatoma and adhesive otitis. This is in complete agreement with the pathogenesis of these diseases, in which poor tubal function is of decisive importance.

After the operation, tubal function improved to some extent (Tos, 1974), but half the patients will go on having problems because of reduced tubal function and one-quarter will develop retractions of the ear drum and/or retraction pockets. The frequency of retractions differed widely between the various diseases and tallied with the tympanometric findings. Tubal function was not further reduced after a follow-up period of 4 years, and apparently the frequency of retractions also did not increase after that time, except in ears with a history of cholesteatoma.

The present paper is a preliminary report, and for lack of space it is not possible to discuss here the numerous aspects presented by this material. In particular, the formation of retraction pockets in cholesteatoma must be analysed in greater detail and related to the various surgical methods and to the duration of follow-up.

ZUSAMMENFASSUNG

Bei 512 Ohren mit verschiedenen Mittelohrkrankheiten wurde Mittelohrdruck mindestens zwei, höchst 10 Jahre nach der Tympanoplastik gemessen. Die Befunde wurden zur Observationszeit Retraktionen des Trommelfells und/oder im Atticus relatiert. Die Tympanometrieverhaltnisse waren am besten bei Sequelae nach der Otitis mit trokenen Perforationen, am schlechtesten bei Cholesteatomen und adhasiver Otitis. Die Tubenfunktion verschlechtert sich nicht 4 Jahre nach der Operation. Es wurde ein hoch signifikanter Zusammenhang zwischen Mittelohrdruck und Haufigkeit der Retraktionen gefunden. Bei den meisten pathologischen Zustanden – außer Cholesteatom – nahmen die Retraktionen 4 Jahre nach der Operation nicht zu.

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MASTOID OBLITERATION

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Abstract Mastoid obliteration is recommended as a routine procedure in all mastoid surgery Bone chips from the mastoid tip, bone paté from the cortical bone and lyophilized dura can all be employed effectively to fill the epitympanic space and Trautmann s triangle The former annulus is reconstructed using lyodura and periost bearing bone and the canal wall is fortified and the cavity filled with the meatally based postauricular musculoperiosteal flap Any remaining cavity is filled with bone chips and bone pate

Mastoid obliteration was introduced by Mosher (1911) to promote healing of the post auricular cavity Leaving a superior pedicle, he cut the flap at the back of the auricle, which meant that all facial nerve branches to the flap were invariably severed and which, in addition, resulted in a rather poor blood supply from the thin upper pedicle A better flap was devised by Popper (1935) who employed a horse-shoe formed, periosteal flap with a broad pedicle towards the auricle Popper's aim was not to obliterate the cavity but rather to provide a living lining over bone in the posterior part of the cavity

The flap I have advocated, first for mastoid obliteration (Palva, 1962) and later also for ear canal reconstruction (Palva, 1963), has the same form as Popper's flap but includes all subcutaneous tissues from the retro auricular area down to the bone, preserving the facial nerve branches intact inside the broad meatal pedicle As to length and width, the flap can be tailored as desired to fill large cavities too The incision should always be made 1–1½ cm behind the postauricular fold in order to facilitate the liberation of the flap backwards

Postoperative follow up of our first series (Palva et al , 1965) showed that in the ears which became adhesive, the flap had yielded posteriorly, which resulted in an ear canal that was wider than normal, and sometimes so wide as to resemble a small cavity In a few cases, on account of the relative narrowness of the lateral part of the ear canal, pockets formed which were difficult to clean from wax and accumulating keratin from the skin To prevent this, some modifications have subsequently been made to obliterate the whole original cavity more effectively

The present technique utilizes periosteum attached bone chips from the mastoid tip, bone paté drilled from healthy cortical bone, and lyophilized dura The primary prerequisite for obliteration is thorough mastoid bone work in which the sigmoid and dural laminae are exposed and the semicircular canals deskeletized

Particular attention is directed to the epitympanic area and to the lower part of the removed bridge The primary concern is to ensure total removal of cholesteatoma epithelium from both the mastoid and the middle ear Very often the posterior canal wall has to be drilled down to the level of the lowermost part of the tympanic cavity along the facial nerve canal The only areas where at primary surgery remnants of cholesteatoma epithelium may be left intentionally are the oval window niche and an area covering a labyrinthine fistula Unintentionally it may be left in a deep facial sinus In case of doubt, second stage surgery is performed 3 to 12 months later

The first stage of the obliteration technique is to place 2 to 4 pieces of lyophilized dura on top of each other in the epitympanum and

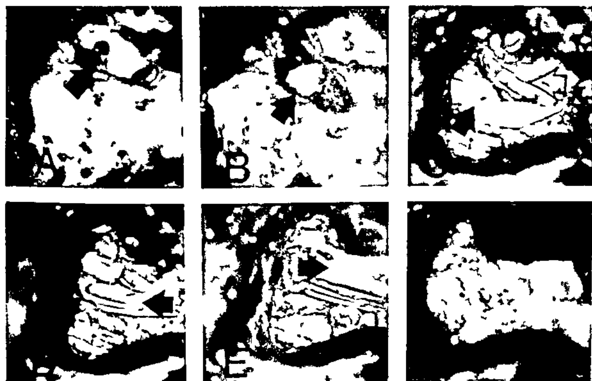


Fig 1 Various stages of mastoid obliteration surgery (A) Stage before the start of reconstruction cholesteatoma having been removed Arrow indicates a projecting stapes (B) Homograft incus columella (*arrow*) surrounded by gelfoam the short process projecting to the umbo area (C) Fascial graft has been turned behind the canal skin (*open arrow*) the epitympanum and facial nerve area have been raised with lyodura (*arrow*) and Trautmann s triangle is filled with bone chips and bone pate (D) Further pieces of lyodura have been applied to raise the new annular rim area (*arrow*) Filling of the epitympanic

on the facial nerve canal until the level of the lateral semicircular canal is reached Larger curved pieces of lyodura are then applied extending from the epitympanum to the lowest part of the removed canal wall bone In this way an attempt is made to raise the point at which the grafted drum is to be attached to the level of the former annulus

At this stage filling of the posterior part of the cavity Trautmann s triangle in particular is started For this purpose small bone chips and bone pate in ampicillin solution are used alternately and filling is continued until the level of the lyodura annular reconstruction is reached Drum reconstruction is now made with fascia or with cialit preserved dura which is applied to the undersurface of the anterior drum remnant on top of the ossicular reconstruction while the remaining larger part of the graft is allowed to lie temporarily on the bone chip–bone pate filled cavity In applying the graft it is important not to shift it along the ossicles but to fit the proper area directly on top of the ossicles and to roll the anterior double turned end of the graft to its site in the tympanum This speeds up the surgery as the columellar graft will not become dislodged during the procedure

The next step is to cover the tympanic part of the graft with a round cigarette paper patch on top of which some 6 to 10 small gentamycin ointment impregnated gauze strips are placed At this stage the retractor is released and by holding the flap with forceps

pulling it backwards, two gauze strips are introduced into the ear canal This packing should have the shape of the original ear canal and the canal skin is carefully smoothed along the packing A small open slit remains at the bottom of the canal and in order to close completely all connections from the canal to the cavity, the remaining part of the graft is lifted up so as to cover the whole of the posterior side of the canal skin

When the cavity is examined at this stage, it is noted that the epitympanum opens directly forwards and, if left unattended, it will form an unnecessary widening of the upper ear canal wall To acoid this, the whole area is filled with 2–3 mm wide and 1 to 2 cm long pieces of lyodura until they extend as far posteriorly as the gauze-filled rounded ear canal At primary surgery, pieces of periosteum covered bone chips are cemented on to the entire posterior canal wall with bone pate, and the musculo periosteal flap is placed posteriorly to this reconstruction If any further free space remains, additional bone chips and bone pate are used, particularly in the lowermost part of the cavity, to fix the flap tip in its proper position

In revision surgery when the skin used to reconstruct the posterior canal wall is often defective or in poor condition, the soft canal wall is further fortified with large pieces of lyodura and not with bone chips and bone paté which then should be used behind the flap Even if the lyodura in the course of healing should become bare, it will soon be overgrown by new skin, whereas exposed bone chips would sequestrate and have to be extracted Also, because temporalis fascia had generally been used at primary surgery, cialit preserved dura should now be available Otherwise the principles and prognosis of primary and revision surgery are fairly similar

The reasons for my increased use of lyodura in canal wall reconstruction are its total lack of irritation, and its being as well tolerated as the body's own collagen In the middle ear too it also has many applications, such as covering denuded surfaces and steadying ossicular reconstructions Use of bone chips with periosteum and bone pate results in the formation of new, healthy bone

Finally, a comment should be made on the objections raised now and again against obliteration in favour of large, air containing mastoids The theoretical calculations of various resorption rates have no application whatsoever For a successful result a functioning Eustachian tube is essential It does not matter in the least in the long run whether an extra volume of some 3 to 5 ml is added to the original 1 5 ml middle ear space in cases of non functioning Eustachian tubes and the total absorption of air delayed a week I would rather claim that if the patient's Eustachian tube is capable of clearing all the accumulated blood from this 3 to 5 ml extra volume, it does not have the least difficulty in maintaining a normal air pressure in the middle ear That the middle ear space is sufficient—as it is—to provide the necessary air reservoir has been fully substantiated during the period of fenestration surgery for otosclerosis, and is frequently seen in patients who have a non pneumatized temporal bone

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CAN AN OPERATION ON A DEAF EAR BE DANGEROUS FOR HEARING?

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Abstract After a simple or modified radical mastoidectomy the hearing of the contralateral ear was followed by pure tone audiometry in 55 patients In 12 patients a sensorineural—mostly high tone—hearing loss of at least 20 dB was found in the contralateral ear In six cases this was persistent The number of patients with hearing loss increased with increasing operation time The cause of this hearing loss must be the noise of the bone-cutting burr

Sensorineural hearing loss in chronic ear surgery has mostly been connected with excessive movements of the ossicles (Schuknecht & Tonndorf, 1960, Palva et al, 1973, Smyth 1977) Also the noise or even thermal effect of drilling has been found to traumatize the cochlea (Montmollin, 1960, Paparella 1962 Helms, 1976, Kylen & Arlinger 1976, Rice, 1976, Kylen et al, 1977*a*, Call 1978) The part played by each of these factors is not clearly described in the literature

The noise of drilling in the ear under operation may exceed 100–130 dB (Helms 1976 Kylen & Arlinger, 1976 Soudijn 1976) and is about 6–8 dB less in the contralateral ear These noise levels can cause permanent damage after an exposure time of about half an hour (Miller, 1974)

To obtain information especially about the acoustic trauma caused by drilling we have followed the hearing of the contralateral ear after chronic ear surgery

MATERIAL AND METHODS

The material consisted of 55 unselected patients subjected to a simple or modified radical mastoidectomy The patients were operated on during the years 1975 and 1976 The duration of the operation was less than 3 hours for 22 patients and 3 hours or more for 33 others The duration of the operation was used as suggestion of a shorter or longer drilling time The patients were operated on by both senior and resident surgeons The mean age of the patients was 35 (range 14–63) years

On the day before operation the pure tone threshold was measured The test was repeated on the non operated ear daily during the first 10 days after operation, while the patients were still in hospital If any changes in the hearing of the contralateral ear were found, the test was repeated on both ears during the control visits to the Outpatient Department 3, 8, 16 and at least 26 weeks after operation

RESULTS

Table I shows the results of measurements during the first postoperative week, when the changes of hearing were maximal The material was divided into two groups according to the duration of operation, less than 3 hours, and 3 hours or more Though hearing was regarded as worsened if the threshold in one or more frequencies had fallen by 20 dB or more, even slight changes between 10 dB and 20 dB are shown as well The losses were always purely sensorineural and found mainly in the frequencies 3–8 kHz

The number of persistent hearing losses after a minimum observation time of 26 weeks are shown in Table II

In 4 cases the hearing loss extended to the

Table I *Hearing loss measured by pure tone audiometry during the first postoperative week*

Maximum hearing loss	Operation		Total
	<3 hours	≥3 hours	
10–15 dB	3	7	10
15–20 dB	4	5	9
20–25 dB	0	5	5
25 dB	1	6	7
Total	8	23	31

low frequencies Fig 1 shows an example of this The modified radical mastoidectomy in this case lasted for 5 hours This hearing loss recovered completely in 2 weeks

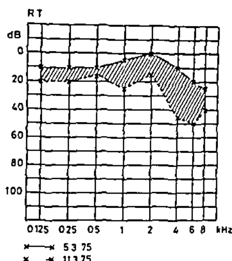


Fig 1 Preoperative and postoperative pure tone thresholds of the contralateral ear of an 18 year-old man

DISCUSSION

Acoustic trauma caused by the bone cutting burr in chronic ear surgery may be studied by observing the hearing of the contralateral ear In this way the possible effect of direct manipulation of auditory ossicles or middle ear windows is excluded

It is well established in the present study that chronic ear surgery may cause sensorineural hearing loss also in the contralateral ear Thus operation even on a deaf ear may be dangerous for hearing

Most of the hearing losses were high tone ones as in typical acoustic trauma In 4 cases a low tone hearing loss was also observed This phenomenon, when found in the operated ear, has been assumed to result from direct manipulation of the ossicular chain (Paparella, 1962, Kylen et al, 1977*b*) The reduced or absent function of stapedius reflex during general anesthesia may account for the low tone hearing loss Zakrisson & Borg (1974) have stated that stapedius reflex in man may protect the ear against auditory fatigue, probably also against premanent injury within the low frequency region

Table II *Persistent hearing loss measured by pure tone audiometry*

Maximum hearing loss	Operation		Total
	<3 hours	≥3 hours	
10–15 dB	1	1	2
15–20 dB	1	1	2
20–25 dB	0	3	3
25 dB	1	2	3
Total	3	7	10

Hearing losses observed in the present study were mostly transitory and very slight However, permanent losses were also found when the drilling time was prolonged In the prevention of acoustic trauma in chronic ear surgery the minimizing of drilling time is the most important thing Variations in rotation speeds or types of cutting burr do not significantly alter the noise generated by the burr (Kylen et al, 1977*a*) These facts again stress the importance of cadaver training in chronic ear surgery Most of the lengthy operations in the present series were done by residents

Another way of preventing the hearing loss may be found in the observation of Joglekar et al (1977) who stated that temporary threshold shift diminished more quickly if the subject breathed pure oxygen—or even better, carbogen (95% oxygen and 5% carbon dioxide) This effect was observed with both post stimulatory and perstimulatory gas inhalation Thus, taking care of optimal oxygenation dur

ing the operation and carbogen inhalation after a lengthy chronic ear operation may be valuable for prevention of acoustic trauma caused by drilling

ZUSAMMENFASSUNG

Das Gehor des kontralateralen Ohres war nach der Radikaloperation des Mittelohres mit der Tonaudiometrie in 55 Patienten untersucht In 12 Patienten war ein postoperativer sensorineuraler Hörverlust über 20 dB entdeckt Dieser war in den meisten Fällen nur in den hohen Frequenzen lokalisiert In sechs Fällen war dieser Hörverlust dauernd Die Ursache des Hörverlusts muß das Gerausch des Bohrers sein

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HEARING GAIN CALCULATIONS AFTER STAPEDECTOMY

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Abstract A comparison of the postoperative air–bone gaps to pre and postoperative bone conduction (BC) was made in two groups of patients with otosclerosis 18 cases with poor preoperative BC thresholds and 15 cases with good preop thresholds The gaps to the postoperative BC showed less variation than the gaps to the preop BC threshold The postop BC may therefore serve as a more stable and natural reference when calculating a postoperative air bone gap

Stapedectomy carried out by various techniques has for the last two decades been successfully employed in patients with otosclerosis Several authors have reported good postoperative hearing in about 90% of the cases (House, 1962, Shea 1958, Schuknecht, 1971) Patients with an unimpaired preoperative cochlear function present few problems when calculating the postoperative hearing gain Cases with a poor preoperative bone conduction (BC) threshold often show considerable BC improvement postoperatively and, with the present method of calculation, an over closure of the air–bone gap This phenomenon has been known for a long time and was pointed out in 1959 by Rosen et al In view of this, we find it most surprising that the standard procedure when reporting on hearing results after stapedectomy (and after tympanoplasty for chronic ear disease) is to compare preoperative BC with postoperative air conduction (AC) thresholds a postoperative gap of less than 10 dB being considered a success

This method of calculation is widespread in the literature and was recommended by the Committee on Nomenclature in Chronic Ear Disease and the Otosclerosis Study Group (Austin, 1971) It is obvious that with this method a postoperative air–bone gap of 10 dB reported as a success may well be a failure especially in cases with poor preoperative BC thresholds

In connection with electrocochleographic studies of patients with severe otosclerosis we found a high incidence of postoperative 'over closures' of the pure tone audiometry air-bone gap We present this material as a contribution to the continuing discussion on how to evaluate the postoperative hearing gain after surgery for otosclerosis and chronic otitis media

MATERIAL AND METHODS

Two groups of patients were selected The first comprised 18 patients with an impaired BC threshold either as a pronounced Carhart notch or a general depression of the threshold Pre- and postoperative audiometry was performed by the same audiometrician using the same audiometer One surgeon operated on all these patients using the same technique (House, wire and fascia) All cases were considered successful The second group comprised 15 patients with a good preop BC threshold, i e Carhart notch less than 10 dB These patients were found by scrutinizing the records of all cases of stapedectomy (400) for the last 3 years Audiometry was not standardized beyond clinical practice in this group Two surgeons using the same technique as above were involved From the audiograms the air–bone gaps for 0 5, 1 2 and 4 kHz were calculated using both pre and postoperative BC thresholds, Student s t test was used for group and method comparisons

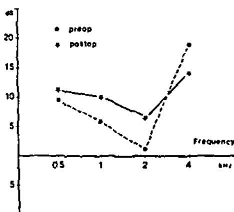


Fig 1 Pre and postoperative BC thresholds relative to postoperative AC thresholds in 15 cases with good preoperative BC. Mean values.

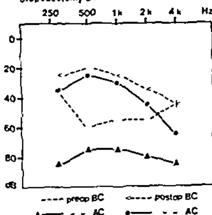


Fig 3 Pre and postoperative pure tone audiograms in a case with a poor preoperative BC threshold.

RESULTS

By making postop AC threshold a reference level, the mean gap for each frequency and each method of calculation is demonstrated. Fig 1 shows this for the 15 patients with good preop BC thresholds and Fig 2 illustrates this for the 18 patients with poor preop BC thresholds. At 1 and 2 kHz the two methods for calculating the air–bone gap give significantly differing results in both groups of patients ($p<0.05$). At 500 Hz and 4 kHz the differences are not always statistically significant. For the average of the three speech frequencies 0.5, 1 and 2 kHz, the two methods give significantly differing results in both groups: $p<0.05$ in the good group and $p<0.005$ in the group with poor BC thresholds.

Except for 1 kHz, no significant difference was found between the two groups of patients when the gap postop AC to *postop* BC is considered. However, evaluating the gap postop AC to *preop* BC yields significantly differing results ($p<0.05$) for the two groups of patients except at 4 kHz. Thus it seems as if

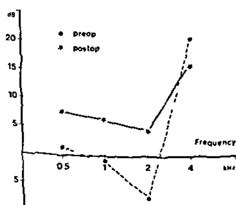


Fig 2 Pre and postoperative BC thresholds relative to postoperative AC thresholds in 18 cases with poor preoperative BC. Mean values.

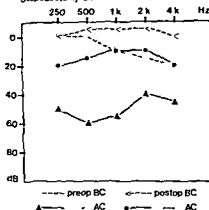


Fig 4 Pre and postoperative pure tone audiograms in a case with a good preoperative BC threshold.

the gap to the preop BC depends on the level of the preoperative BC thresholds On the other hand the gap to postoperative BC seems to be independent of the preop BC threshold level

DISCUSSION

In Figs 1 and 2 we find that cases with good preop BC do not show an overclosure of the air–bone gap, which, however, is shown for the 1 and 2 kHz frequencies in cases with poor preoperative BC if this threshold is used as a reference

Fig 3 shows the pre- and postoperative pure tone audiograms in a case of a poor preop BC Postoperatively we have an obvious overclosure of the gap to the preop BC, while to the postop BC, the AC lies within 10 dB The remarkable thing is, however, what might *have happened if the postoperative AC had* ended up 10 dB below the preop BC With present procedures (AC–BC preop) the case could have been reported as a success even if the AC thresholds had been 35 dB below the present one

It is also interesting to note by direct comparison of Figs 1 and 2 that the gaps to postoperative BCs seem to vary less than the gaps to the preoperative BCs and it is thus quite evident that in both groups of patients the postoperative BC forms a more natural and stable reference when calculating a postoperative air–bone gap In addition it is quite clear from these two figures that not only is there a postoperative BC improvement in the poor cases, it also exists in the good ones When the preoperative BC is used as a reference the gap is closed within 10 dB in the 0 5–2 kHz frequencies but when the postop BC is used, this is not the case This fact is also illustrated in Fig 4, showing the pre and postoperative audiograms in a patient with an initially good BC After stapedectomy the gap to the preoperative BC is well within 10 dB (0 5–2 kHz), to the postoperative one it is 16 7 dB This illustrates the need for a change in the rules concerning what is acceptable as a postoperative air–bone gap if the postoperative BC is to serve as a reference threshold

Whatever the reason behind a poor BC in advanced otosclerosis might be, the postoperative BC undoubtedly represents the true cochlear function better than does the preoperative one, which gives further support for the use of the postoperative BC in hearing gain calculations after surgery Of course this presupposes a parallel account of pre- and postoperative speech discrimination scores in order to evaluate any cochlear losses

The whole problem is a very complex one and we do not pretend to have a solution Many techniques are used in stapedectomy today, all giving excellent results with the present method of calculation We feel that the only way to improve the existing surgical techniques is to narrow the definition of success *in postoperative air–bone gap evaluation*

ZUSAMMENFASSUNG

An zwei Gruppen von Patienten die an Otosklerose litten davon 18 Falle mit schlechter praoperativer Knochenleitungsschwelle (KLS) und 15 Fälle mit guter praoperativer KLS wurden Vergleiche zwischen den postoperativen Schalleitungskomponenten auf der praoperativen KLS einerseits und auf der postoperativen KLS anderseits aufgestellt Die postoperativen Schalleitungskomponenten zeigten weniger Schwankungen wenn sie auf die postoperative KLS bezogen wurden Die postoperative KLS mag deshalb als eine stabilere und naturlichere Richtlinie gelten wenn es um die Berechnung der postoperative Schalleitungskomponente geht

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BLEOMYCIN AS ADJUVANT IN RADIATION THERAPY OF ADVANCED SQUAMOUS CELL CARCINOMA IN HEAD AND NECK

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Abstract Since 1969 Bleomycin (BLM) has been used in three different ways at the Radium Centre in Copenhagen First BLM given as the sole treatment led to complete regressions in 12% of 138 patients (12) Secondly BLM was used as simultaneous adjuvant in radiation therapy for 86 previously untreated patients but 66% developed mucositis which disrupted the treatment In a third period BLM was therefore combined sequentially with radiation administered for 2 weeks prior to radiation therapy to 142 patients The tumour shrinkage achieved with pre irradiation BLM was very pronounced in 38% of cases 101 patients with T3 tumours have been observed for a minimum of 3 years The medium survival time 47 months for those sequentially treated patients responding pronouncedly to BLM was greater than for the remainder of the sequentially treated patients 16 months ($2p=0.010$) and for the patients treated simultaneously with BLM 15 months ($2p=0.013$) The prognostic value of the degree of shrinkage achieved with preirradiation BLM treatment is discussed

Bleomycin, BLM, isolated by Umezawa (14) is an antitumour antibiotic BLM has the special advantage of concentrating in squamous cell cancer (15), thus avoiding unwanted immunosuppression (1) and bone marrow toxicity (11) It concentrates in skin, lung, liver, spleen and bone marrow (15), but is inactivated in bone marrow and liver and toxicity is therefore limited to skin and lung in which the induced fibrosis may be lethal if untreated BLM is excreted with the urine in active form and normal kidney function is a prerequisite to treatment

The mechanism of the cytostatic effect has been reviewed by Umezawa (16) An interesting feature is the similarity of action of BLM and ionizing irradiation On the molecular level, both treatments can produce double-strand breaks in DNA molecules (5) This may explain why cells synchronized in vitro exhibit least survival in mitosis, G2, and S phase in that order, when exposed to BLM or irradiated (2 13) Clinically the rapid tumour shrinkage and localized mucositis in BLM-sensitive tumours closely mimics the events in tumours responding during radiation therapy In solid transplanted mouse tumours, BLM given 2 hours before irradiation acts synergistically (7), possibly because BLM hinders the repair of sublethal radiation damage (17)

In clinical work, Ischikawa's good results with BLM in the treatment of cancer of the penis in 1965 (6) prompted several investigations in Europe Cooperative screenings begun in Denmark 1969 (11) and in EORTC (4) disclosed that the best response was obtained in squamous cell cancers of the head and neck In Copenhagen, BLM as the sole remedy led to complete regressions in 25% of 107 neck tumours (12) Furthermore, BLM has been used as an adjunct to radiation therapy in two consecutive groups of patients In the first treated group (I), BLM was given simultaneously during radiation therapy, and in a second, sequentially treated group (II), BLM was given for 14 days before the start of radiation therapy The results obtained in T3 tumours in these two groups are reported here

MATERIALS AND METHOD

From March 1970 to July 1974, 101 previously untreated patients referred to the Radium Centre of Copenhagen for head and neck can-

Table I *The distribution according to origin and frequency of lymph node metastases in 101 T3 tumours treated either by simultaneous or sequential combination of BLM and radiation (≥1 500 reu)*

Site of primary	I Simultaneous BLM					II Sequential BLM				
	N0	N1	N2	N3	Total	N0	N1	N2	N3	Total
Larynx	10	–	–	1	11 (33%)	25	1	–	–	26 (38%)
Oral cavity	5	5	3	–	13 (40%)	13	4	5	3	25 (37%)
Pharynx	3	2	1	3	9 (27%)	7	5	–	5	17 (25%)
	18 (55%)	7 (21%)	4 (12%)	4 (12%)	33 (100%)	45 (66%)	10 (15%)	5 (7%)	8 (12%)	68 (100%)

cers classified as T3 (UICC, 1972), received BLM as an adjunct to radiation therapy Group I, 33 patients, received simultaneous and group II, 68 patients, received sequential treatment Patients were not randomized between the two modes of treatment, which were used in different periods There was, however, some overlap because the two modes developed through a sequence of efforts, first to reduce toxicity and then to advance BLM from a palliative treatment in recurrent cancer to become part of the primary treatment of previously untreated patients

All patients had biopsies taken, showing invasive squamous cell cancer—except for 6 described as carcinoma in situ These are included, since obvious gross tumour was present and 4 developed invasive carcinoma High differentiation, i e parakeratosis and/or monocellular keratinization, was described in 84% The age of the patients varied considerably The mean age in group I was 61 4 years (49–86) and in group II, 63 2 (47–84) Men dominated in group I, 82%, compared with 68%, in group II The proportion of biopsies described as highly differentiated was 82% in group I and 87% in group II

Table I shows the distribution of the 101 T3 tumours according to treatment, as well as site of origin and frequency of lymph node metastases There is a slight predominance of laryngeal tumours in group II, and of patients with N1 and N2 lymph node metastases in group I

Table II *Mean values of doses of BLM and radiation in 101 patients with T3 squamous cell cancer in head and neck regions, according to response to either simultaneous or sequential BLM adjuvant treatment*

Group of results	Modality of BLM and/response		Number of pats	BLM Dose mg mean	≤100	≥200	Radiation dose CRE reu
A Complete regression without recurrence	Simultan	I	15	124	53%	13%	1 720± 75[a]
	Sequential	IIa[b]	16	115	25%	–	1 767±151
	Sequential	IIb[c]	14	150	7%	14%	1 769±450
B Operated free of remnant/recurrent	Simultan	I	6	103	50%	–	1 630± 85
	Sequential	IIa	4	124	25%	–	1 710± 57
	Sequential	IIb	8	167	–	13%	1 746±255
C Died of cancer	Simultan	I	12	156	33%	25%	1 700+112
	Sequential	IIa	6	169	17%	33%	1 799±140
	Sequential	IIb	20	140	20%	10%	1 700± 96

[a] Standard deviation
[b] Tumours shrinking pronouncedly after BLM (+)
[c] Tumours shrinking minimally after BLM (+/–)

Table III *Comparison of results in T3 tumours treated either by simultaneous or sequential combination of BLM and irradiation (≥1500 reu)*

Modality of BLM adjuvant	Total number pats	Patients free of tumour at end of observation: No further treatment A	Operated rec/remn B	Total free of tumor A+B	Local failures: Patient died with cancer C
Simultaneous I					
BLM + radiation	33	15 (45%)	6 (18%)	21 (63%)	12 (37%)
Sequential II					
BLM + radiation	68	30 (44%)	12 (18%)	42 (62%)	26 (38%)
Shrinkage prior to radiation					
IIa +	26	16 (62%)	4 (15%)	20 (77%)	6 (23%)
IIb +/−	42	14 (33%)	8 (19%)	22 (52%)	20 (48%)

Bleomycin treatment

The schedule used in Japan (6) of 30 mg BLM given intravenously twice each week to a total dose of 300 mg was only used when BLM was given alone. From March 1970 the dose was reduced to 10–15 mg given intramuscularly 2–3 times each week, which reduced the frequency of lung changes from 22 to 8% without any reduction in complete regressions (12). From January 1972 the dose of BLM was weight adjusted to 0.7 mg/kg/week. The BLM was administered in sterile saline containing 1 mg BLM per ml the i.m. injection of which caused neither pain nor local inflammation. In group I, BLM was injected ½ to 1 hour before irradiation, and only during part of the radiotherapy regime. Thus 6 patients started BLM from the first day of irradiation, 6 in the second or third week, 16 in the fourth to sixth week and 5 had BLM only during the last part of radiation therapy. Twenty one patients developed mucositis disrupting the irradiation.

In group II, the tumour shrinkage by the preirradiatory BLM was evaluated on the first day of irradiation, but BLM treatment was continued for about one week during radiation therapy in 53 of the 68 sequentially treated patients.

In Table II the mean values of BLM doses are given for group I, for the simultaneously treated patients and for group II subdivided into group IIa, patients whose tumours shrank very pronouncedly during preirradiation BLM treatment (++), and group IIb, with less or no shrinkage of the primary tumour (+/−). The BLM doses varied considerably in all subgroups. The standard deviation of the mean varied from 21 to 60%, and therefore in each subgroup is shown the fraction given 100 mg of BLM or less and that given 200 mg BLM or more.

Radiotherapy

Radiation was supplied by ^{60}Co equipment. Treatment schemes were based on isodose curves, and tumour dose calculated as the minimum dose within the tumour bearing volume. The daily tumour dose ranged from 160 to 200 rads. The total dose aimed at the maximum tolerance of normal tissues. In the group given simultaneous BLM, severe mucositis often required pauses in the irradiation, which was resumed after the reaction had subsided. Because of these irregularities, the biological equivalence of each irradiation schedule was evaluated using calculation of the cumulative radiation effect, CRE, according to Orton's modification of the NSD concept and given in reu units (9). No attempt was made to replace part of the radiation by BLM. Most of the radiation dose could be considered curative,

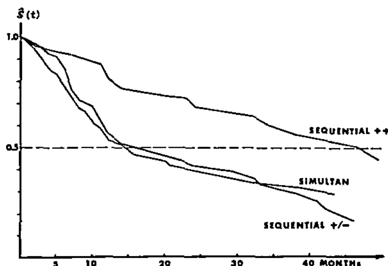


Fig 1 The survivor function as calculated by Kaplan Meier for Group IIa sequentially treated patients with pronounced preirradiation shrinkage of tumour (++) group IIb with less preirradiation shrinkage (+/-) and group I patients treated simultaneously with BLM and radiation

i e ≥1700 reu Because of severe mucositis some patients in group I had to decline further treatment after 1500–1650 reu Since some tumours regressed completely without recurrence after 1500 reu, all patients given this dose or more are included in both treatment groups The mean values of CRE and their standard deviations, given in Table II, vary less than for BLM

RESULTS

All patients have been observed for a minimum of 3 years for primary tumours (Table III) divided into three subgroups according to treatment results (A) complete tumour regression without recurrence, (B) remnant or recurrent tumour operated and remaining free of tumour, and (C) patients dying of cancer In order to evaluate the effect of the treatments as such, the fraction that group A constitutes of the total, A+B+C, is calculated For lymph nodes, N1+2, this fraction is 6/11 in group I and 4/15 in group II, and for N3 1/4 in group I and 3/8 in group II

Table III shows the results obtained in the primary tumours There is no statistically significant difference in the crude results between these three groups However since more than half of the patients have died in all three groups, the median survival has been calculated by Kaplan Meier survivor function (8), and the difference between survivor functions is calculated using log rank test The results are shown in Fig 1 The median survival in group IIa is 47 months, which differs from the median survival of 16 months in group II b (2p 0 010) and that of 15 months for group I (2p 0 013)

In order to rule out the possibility that the differences in median survival times might be due to different treatment doses, sex ratio, or fractions of low differentiated tumours in the groups, the following analysis was made

The doses of BLM differed greatly, demonstrating the pronounced individual variation in tumour effect and tendency to develop mucositis In group I the fraction of BLM doses of 100 mg or less was thus the largest in all subgroups (Table II) Among patients with lasting regressions (A), the total dose of BLM in tumours shrinking pronouncedly, Ia, (++) was 115 mg compared with 150 mg in patients with tumours shrinking less, IIb, (+/-) This demonstrates that in tumours responding, the shrinkage is rapid

The doses of radiation varied little and it is of special interest that the mean dose of 1772± 123, calculated for tumours shrinking pro-

nouncedly (IIa), and those shrinking less (IIb), leading to complete lasting regressions (A), or death from cancer (C) had a standard deviation of only 7%

The influence of small radiation doses, female sex, and low differentiation, is best evaluated by calculating the fraction which patients with complete and lasting regressions (A) constitute of the total of these patients within each treatment group I, IIa, and IIb

The results obtained with radiation doses of 1 500–1 650 reu are similar to the overall results (Table III), since the ratio of patients with lasting complete regression (A) constitute 7/12, 3/4 and 3/5 of the total of these patients in groups I, IIa, and IIb

The fraction of females with lasting complete regressions, A, constituted 6/6, 7/10 and 4/12 of all females (A+B+C) in groupe I, IIa and IIb respectively The females thus seem to fare better than males by simultaneous treatment, groupe I (p 0 025)

The fraction of low differentiated tumours with complete lasting regressions (A) compared with the total number of these tumours (A+B+C) was 4/6 4/5 and 1/4 in groups I, IIa and IIb and do not seem to differ from the overall results (table III)

DISCUSSION

The statistically significant greater median survival time of 47 months for patients with pronounced preirradiation tumour shrinkage in group IIa compared with 16 months for the patients with less or no shrinkage in group IIb was found to be independent of the fractions of patients in the two groups given doses of 1500–1 650 reu, the fractions of females and those of low differentiated tumours The treatment results obtained in these sequentially treated groups are thus comparable, since they were treated in the same period of time with BLM doses in favour of group IIb and with almost identical radiation doses Whether the simultaneously treated patients in group I can be considered as historical controls is open to discussion The inferior results obtained in this group can hardly be attributed to the slight difference from group II as to the fraction of tumour sites of origin (Table I), but rather to the lack of improved results that can be obtained by adding BLM simultaneously to radiation therapy The only randomized study carried out in EORTC, resulted in no difference as the fraction of immediate complete regressions was 67% of 93 patients given radiation only and 68% of 87 patients given simultaneous BLM and radiation therapy (5) In the present series no attempt was made to replace part of the radiation by BLM, and it is open to discussion whether the 13 patients with lasting complete regressions of the 21 given 1 500–1 650 reu had any benefit from the BLM treatment or merely represented the most radiosensitive tumours

It is a commonly shared opinion that the exophytic and least necrotic tumours respond best to BLM as well as to radiation The better prognosis found here for patients with tumours shrinking pronouncedly before radiation, may therefore merely reflect a selection of patients with the most radiosensitive tumours The radiobiological value of irradiating a grossly diminished tumour is obviously the advantage of irradiating a better oxygenated tumor

More intensive preirradiation chemotherapy could possibly increase the frequency of pronouncedly shrinking tumours Thus P Clifford (3) employing sequential 3 drug chemotherapy alternating with radiation therapy seems to obtain better survival rates than those obtained here, but has not mentioned the frequency of preirradiation tumour shrinkage Future attempts at improving the results of combined chemo-radiation therapy could possibly take advantage of the prognostic value of observing the degree of preirradiation tumour shrinkage, as found here

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INTRA-ARTERIAL CHEMOTHERAPY OF MALIGNANT HEAD AND NECK TUMOURS WITH SUPERSELECTIVE ANGIOGRAPHIC TECHNIQUE

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Abstract A technique is described for repeated superselective angiographic infusion of anticancer drugs into advanced malignant tumours of the head and neck region The technique allows for a high concentration of the drug in the tumour and furthermore it is possible to ensure that the infused anticancer drug adequately reaches the tumour Mitomycin C was given to 15 patients with advanced malignant tumours of the head and neck region In 6 patients a partial remission was seen and in another 5 patients a subjective improvement was reported No complications were observed

Intra arterial chemotherapy of malignant tumours of the head and neck region has been tried for palliation or potentiation of radiotherapy The aim of selective intra-arterial chemotherapy is to expose the tumour tissue to higher levels of the anticancer drug than the marrow and gastrointestinal mucosa (Bertino et al , 1975) Intra arterial chemotherapy in the head and neck region has been given for weeks or months as continuous infusions into the external carotid artery or its branches The technique for this long term infusion therapy consists of a direct operative approach to the external carotid artery system After the surgical placement the intra-arterial catheter is connected to an infusion pump that delivers the anticancer drug continuously into the tumour region (Oberfield et al , 1973) Despite careful supervision by a trained staff this technique can be beset by technical problems and considerable morbidity (Goldsmith & Carter, 1975)

In the present report a different technique for repeated intra arterial chemotherapy of malignant head and neck tumours is described By applying a superselective angiographic technique the anticancer agent is infused during 10–20 minutes into the tumour region After infusion the catheter is immediately withdrawn This report describes the technique and the early results

MATERIAL AND METHODS

Fifteen patients with non resectable malignant tumours of the head and neck region were treated at the University Hospitals of Lund and Malmö by repeated superselective angiographic infusions of mitomycin C between January 1977 and April 1978 There were 3 women and 12 men, with a mean age of 64 years, range 29–76 All patients had advanced tumours and all were unresectable Eight patients had previously been treated with surgery and/or radiotherapy In 7 patients the intra arterial infusion therapy was given prior to radiotherapy The distribution of the 15 malignant tumours on various locations is shown in Table I As can be seen cancer localized to the maxillary sinus and oral cavity dominated the material All but 3 patients had squamous cell carcinomas with varying degrees of differentiation The remaining patients had different types of adenocarcinomas

A selective angiography of the external carotid artery was done under local anesthesia After percutaneous puncture of the femoral artery a catheter was advanced into the external carotid artery and an angiography

Table I *Distribution of malignant tumours treated with superselective angiographic infusions of mitomycin C*

Location	No of tumours
Maxillary sinus	4
Tongue	4
Base of tongue	2
Gum	2
Hypopharynx	1
Nasopharynx	1
Thyroid	1

was performed to visualize the vascular supply of the tumour The catheter was then advanced to a superselective position into or as close as possible to the tumour feeding artery To reduce pain, Metrizamide (280 mg iodine/ml) was used as a contrast medium The position of the catheter tip was frequently checked by fluoroscopy during infusion In some cases, also 3–5 ml aqueous solution of 5% fluorescein dye was injected through the catheter With the catheter tip in an accurate position the tumour region had a yellow coloration on illumination by a Wood s lamp 10–20 mg of mitomycin C (Mutamycin®, Bristol) was diluted in saline to a concentration of 1 mg/ml and injected at a rate of approximately 10 ml/min This total dose was given in portions into as many tumour feeding vessels as possible 1–5 injections were given with an interval of 2 weeks Before each infusion hemoglobin, leukocytes, platelets, liver function tests, electrolytic status and S creatinine were determined

A remission was defined as partial if clinical examination and, in some cases computerized tomography or angiography demonstrated a reduction of tumour size by more than 50% A decrease by less than 50% was defined as no remission Each patient with maxillary carcinoma had three infusions and at every treatment cytological examinations were performed to determine the therapeutic effect on cellular level The functional status was measured by the Karnofsky performance index which is a guideline in assessing the quality of life of the patient It is given in a scale ranging from normal status without any symptoms or evidence of disease (100 points) to a moribund status with fatal processes progressing rapidly (10 points)

RESULTS

In 6 of the 15 patients a partial remission was achieved (Table II) In addition to reduction of tumour size in these patients there was also a relief of pain or pressure symptoms due to the tumour Three of the 6 patients had a maxillary carcinoma and the tumour regression could be followed by computerized tomography In these 3 patients the cytological examinations showed tumour cell necrosis The repeated superselective angiography could sometimes also demonstrate reduction of the tumour size In 5 patients there were no partial remissions but the patients obtained palliation with a marked reduction of symptoms in some cases as much as 40 points as measured by the Karnofsky index Four patients did not demonstrate any response to the infusion therapy Three of these non responders were in poor condition, 20–30 points on the Karnofsky index and the therapy was interrupted after 1–2 infusions

The relation of previous therapy—surgery and/or radiotherapy—to results of infusion therapy is shown in Table III Five out of 6 patients with a partial remission had had no previous therapy After surgery and/or radiotherapy only 1 patient had a partial remission

No patient showed any signs of suppression of the marrow, neurological symptoms or toxic reactions from the liver, kidney and gas

Table II *Response to superselective angiographic infusions of mitomycin C*

Type of response	No of patients
Partial remission	6
Decrease of symptoms	5
No response	4

Table III *Previous therapy in relation to response to superselective angiographic infusions of mitomycin C in 15 patients*

Therapy	Partial remission (No of pats)	Decrease of symptoms (No of pats)	No response (No of pats)
No previous therapy	5	2	0
Previous therapy	1	3	4

trointestinal mucosa One patient suffered a partial alopecia There were none of the complications of intra arterial infusions, which Lundberg (1977) recently has reviewed At the cutaneous puncture site, no infection, leakage or cracks were demonstrated Nor were hemorrhages or thrombosis–embolism observed The catheter tip did not become dislodged or blocked No perforation of the vascular tree was seen leading to inadequate or incorrect infusions In some cases a local spasm developed but after a short withdrawal of the catheter this symptom disappeared Some older patients experienced a transient burning pain in the infused region but no other discomfort

DISCUSSION

The management of advanced malignant tumours of the head and neck region is complicated and requires a multidisciplinary approach Chemotherapy has been introduced as another modality in the combination therapy of these locally advanced tumours

Methotrexate is the anticancer agent most often used in the head and neck region It has been given by systemic routes—intravenously or orally—or by continuous intra arterial infusions According to Goldsmith & Carter (1975) there was a 53% response rate in patients treated by infusion therapy compared with a 40% response rate for systemic methotrexate Donegan & Harris (1972) reported a mortality of 11 4% after continuous infusion therapy, due to technical mishaps with the intra arterial catheters and pumping system In the WHO committee report on chemotherapy of solid tumours (1977) the intra arterial infusion therapy was considered questionable due to the low response rate and the complications Recently Bleyer (1978) has stated that an intra arterial injection of methotrexate only gives slightly higher local tissue levels than intravenous injection He consideres these slightly higher levels as probably insignificant in comparison with the substantial risk at intra arterial infusion

To avoid the technical problems and morbidity associated with the long term infusion therapy a superselective angiographic technique for repeated short term administration of anticancer drugs has been tried By a percutaneous approach a catheter is placed in the femoral artery With this superselective technique it is possible to infuse the anticancer drug adequately into the tumour In the present material there were no complications from the cutaneous puncture site or catheter tip

In the present study mitomycin C, an antibiotic from *Streptomyces caespitosus*, was used as sole agent This anticancer drug, recently reviewed by Crooke & Bradner (1976) inhibits DNA synthesis and has an overall response rate of 20% in malignant head and neck tumours Promising results from infusions of mitomycin C into bronchial arteries in patients with bronchial carcinomas (Hellekant et al , 1978) encouraged us to try this drug in our study

The best results were seen when the infusion treatment was given prior to other therapy If patients receive chemotherapy after surgery and/or radiotherapy, the blood supply will be reduced due to radiation fibrosis or surgical procedures (Sealy & Helman, 1972) An

adequate amount of the anticancer drug may therefore fail to reach the tumour

According to the results of the present series we consider that superselective angiographic technique is a suitable method, free from technical problems or morbidity, for the infusion of an anticancer drug into malignant tumours of the head and neck region A combination of two or more drugs may enhance the therapeutic response A tentative chemotherapeutic combination in advanced malignant tumors of the head and neck region may consist of anticancer drugs given both by superselective angiographic technique and systemically (Mattsson et al , 1977)

ZUSAMMENFASSUNG

Eine Technik fur wiederholte selektive Infusionsbehandlungen mit Cytostatica im Zusammenhang mit einer Angiographie wird fur fortgeschrittene maligne Geschwulste im Kopf und Halsbereich beschrieben Mit dieser Technik erhalt man eine hohe Cytostaticakonzentration in der Geschwulst außerdem kontrolliert man hierbei daß das infundierte Cytostaticum wirkl ch in dem Tumor gelangt 15 Patienten mit malignen Geschwulsten im Kopf und Halsbereich wurden mit Mitomycin C behandelt Bei 6 Patienten trat eine partielle Remission ein und bei weiteren 5 Patienten sah man eine subjektive Verbesserung Es wurden keine Komplikationen beobachtet

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COBALT AND BLEOMYCIN AGAINST CARCINOMAS OF HEAD AND NECK

A Controlled Clinical Study

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In various treatment protocols against squamous cell carcinomas of the head and neck, chemotherapy has been placed together with the classical methods represented by surgery and radiotherapy This is because, although surgery and radiotherapy (or combinations thereof) are the chief tools for treatment of patients with head and neck carcinomas, certain forms of tumour proliferation lie outside the scope of their therapeutical potential

METHODS AND MATERIALS

A Randomized and controlled clinical study, comparing cobalt and Bleomycin (30 Gy/180 mg Bleomycin) with cobalt plus placebo has been carried out in 29 patients with moderately to highly differentiated squamous cell carcinomas of the head and neck

The treatment was given prior to radical surgery and all specimens were examined histologically after serial sectioning Details of the treatment schedule can be seen in Table I

Concomitant with our controlled clinical study, those patients with primary head and neck carcinomas considered to be curable without surgery and those who declined the proposed surgery, received cobalt and Bleomycin therapy The preoperative schedule (Table I) was used during the first 5 weeks, followed by cobalt irradiation up to 45–65 Gy. We now consider the irradiation dose of 45 Gy, used in the combined treatment during the early years, to be suboptimal, and have increased the dose to 65 Gy

RESULTS

In the randomized study no differences in side effects, operative difficulties, or delayed healing were encountered between the groups Tumour response based on clinical and histological criteria was clearly in favour of preoperative treatment with cobalt plus Bleomycin compared with cobalt plus placebo (Figs 1 and 2) By histological examination of serial sections from preoperative specimens, residual tumour was deemed present in a rather

Table I *Time schedule of the preoperative treatment*

Blm Injections of 15 mg Bleomycin or placebo (saline) given i m 1 hour before cobalt irradiation
R Irradiation with cobalt 150 Rad in tumour dose
Total dose Bleomycin 180 mg
Total dose Cobalt 3 000 Rad

	Mon	Tue	Wed	Thur	Fri
1st & 2nd week	Blm+R	R	Blm+R	R	Blm+R
3rd week			No treatment		
4th & 5th week	Blm+R	R	Blm+R	R	Blm+R
6th & 7th week			No treatment		
8th week			Operation		

Table II *Head and neck carcinoma Randomized preoperative treatment with cobalt (30 Gy)+Bleomycin (180 mg) or cobalt (30 Gy)+placebo 1973–75, analysis March 1978 (minimum 2 year follow-up)*

	Cobalt+ Bleomycin+ surgery	Cobalt+ placebo+ surgery
No of pats	15	14
Controlled	87% (13/15)[a]	64% (9/14)[b]
Failures		
Local	0	21% (3/14)
Regional	13% (2/15)	35% (5/14)
Total	13%	56%
Distant metastases	0	14% (2/14)

[a] 1 dead (28 months) 1 dead (42 months)
[b] 1 dead (24 months) 1 dead (10 months)

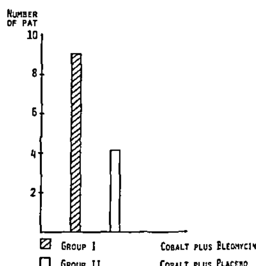


Fig 1 Tumour eliminated or reduced >50% 2 weeks after preoperative treatment (clinical evaluation)

Table III *Squamous cell carcinoma (head and neck) Primary tumours treated with cobalt and Bleomycin[a] (minimum 2-year follow up)*

L Local recurrence R Regional recurrence

Site	Stage	Controlled	Months observed	Failure	Months observed	Salvage surgery
Larynx	I	1[b]	20	1 L	10	1
	II	0	–	2 L	8	1
	III	4	34	1 L	3	1
	IV	0	–	3 L+R	1	0
Oral cavity	III	1	26	3 L+R	17	0
	IV	0	–	1 L+R	2	0

[a] Cobalt 45–60 Gy in tumour dose+Bleomycin 150–200 mg
[b] Pats dead from abdominal cancer without laryngeal tumour

Table IV *Squamous cell carcinoma (head and neck) Primary tumours treated with cobalt Bleomycin and surgery[a] (minimum 2-year follow-up)*

L Local recurrence R Regional recurrence

Site	Stage	Controlled	Months observed	Failure	Months observed
Larynx	II	2	43	0	–
	III	7	34	0	–
	IV	1	29	0	–
Oral cavity	I	2	48	0	–
	II	0	–	1 R[b]	7
	III	2	43	0	–
	IV	0	–	1 R[b]	15

[a] 30 Gy+150–200 mg Bleomycin before surgery
[b] Lingual cancer developing neck metastases Irradiation and surgery for tumour only

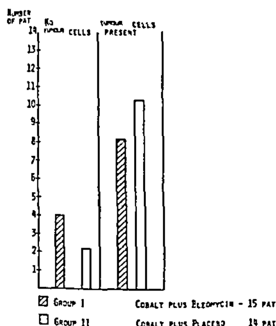


Fig 2 Histological findings in surgical specimens (serial section)

high proportion in both groups (Fig 2) The histological findings also throw doubt on the specificity of the previously reported histo logical changes following Bleomycin treat ment

The follow-up gives further support in fa vour of Bleomycin plus cobalt as preoperative treatment (Table II)

The results from a 2 year follow up of the patients in the open study receiving cobalt and Bleomycin (Table III), do not indicate any improvement as compared with historical data from treatment with irradiation only The failures for laryngeal cancer stages 1 and 2 are disappointing (Table III), whereas the follow-up for patients receiving combined treatment including surgery is encouraging (Table IV)

DISCUSSION AND CONCLUSION

In our controlled study, combined preoperative treatment with cobalt and Bleomycin produced better results than same dosis of cobalt alone However, no conclusions can be drawn concerning the synergistic effect of the combined treatment or superiority to an increased irradiation dose of cobalt only

We consider that Bleomycin's greatest potential in the treatment of head and neck carcinomas lies in its preoperative use in com bination with radiotherapy Radical surgery is essential to achieve optimal tumour control

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CYTOLOGY AND CYTOCHEMISTRY OF ACINIC CELL CARCINOMA

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Abstract In a primary material of 314 epithelial parotid tumours treated at the University Hospital in Umeå since 1958 fifteen (11 ♀ and 4 ♂ patients) were classified as acinic cell carcinomas Four of the female patients were in the age range 16–19 years At the ultrastructural level granulated cells were the predominant cell type in four tumours studied Agranulated cells highly reminiscent of intercalated duct cells were also encountered however [illegible] and analysed for their content of amylase and cyclic AMP The cyto and biochemical findings are discussed and correlated to those observed in normal salivary gland tissue

The degree of malignancy of acinic cell neoplasma varies considerably, a fact which was first seriously discussed by Buxton & co workers (1953) Furthermore, at the histopathological level there seem to be no clearly distinguishable features which permit the pathologist to predict whether or not a certain neoplasm will behave in an aggressive fashion and metastasize (Batsakis, 1974) In most reported series of acinic cell neoplasms, the incidence in females outnumbers that in males by a ratio of 3 or 2 to 1, and Bhaskar (1964) reported on a parotid acinic cell carcinoma which exhibited a marked and rapid growth during pregnancy This led him to discuss a possible influence on tumour growth by endocrine factors Moreover Cox & co-workers (1970), documented an ectopic ACTH production from an acinic cell carcinoma which had spread to regional lymph nodes lungs and the liver

The varied qualities and histopathological features of acinic cell neoplasms some of which have been mentioned above, designate this particular tumour as one of the most puzzling of all salivary gland tumours In the present report some ultrastructural, cytochemical as well as biochemical characteristics of parotid acinic cell carcinomas are outlined

MATERIALS AND METHODS

Since 1958, 314 primary, epithelial parotid tumours have been treated at the ENT and oncologic departments of the University Hospital in Umeå Fifteen of these tumours have been classified as acinic cell carcinomas They were detected in 11 female and 4 male patients Four of the female patients were in the age range 16–19 years The oldest patient was a 78 year-old man Paralysis of the facial nerve, not due to surgical trauma was observed in 2 patients A preoperative diagnosis was established in latter years by cytological analyses of material obtained by fine needle aspiration biopsy Specimens from 4 rather recent patients were procured and processed for ultrastructural cytochemical studies and from 2 other cases, tumour specimens were collected and analysed for contents of amylase as well as cyclic AMP

RESULTS AND DISCUSSION

Cytological examination of aspiration biopsy smears revealed in all the examined tumours a typical cytological pattern The tumour cells appeared in uncharacteristic clusters and showed rather monomorphous nuclei The

cytoplasm was pale, indistinct and finely granulated (Fig 1) In some cases, an admixture of lymphoid cells was encountered in the smears Also at the ultrastructural level, granulated tumour cells were predominant, but agranular, cuboidal cells, lining duct-like lumina were relatively abundant too These agranular cells closely resemble intercalated duct cells, viz they exhibit well-developed junctional complexes, a Golgi complex in a supranuclear position, a relatively sparse rough endoplasmic reticulum and microvilli which project from their luminal surfaces (Fig 2)

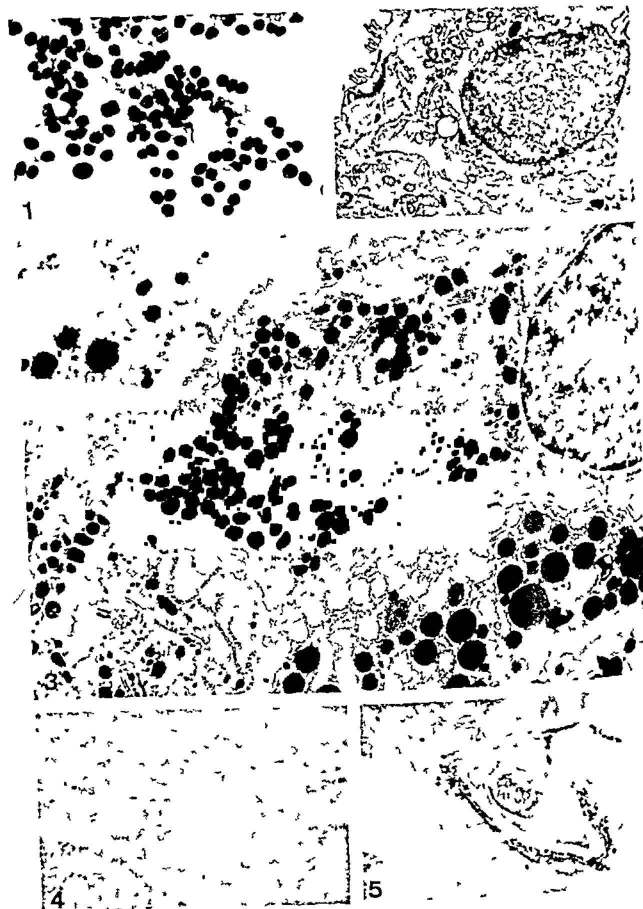
The membrane-bound cytoplasmic granules of the tumour cells varied greatly in appearance as well as size, not only between the different tumours but also between individual cells in one and the same tumour Some granules exhibited a high electron density whereas others were fairly electron lucid, granules with a bipartite substructure were also encountered

It is well known that the granulated tumour cells of acinic cell carcinoma stain well with the PAS technique As a matter of fact, this histochemical staining technique has been considered to be the most reliable technique available for the diagnosis of this tumour In the present investigation we therefore included electron cytochemical techniques to demonstrate periodic acid engendered groups of carbohydrate macromolecules at an ultrastructural level As is the case in numerous glycoprotein synthesizing glandular cells, a positive staining reaction was observed in the cytoplasmic granules as well as in the Golgi complexes of the tumour cells (Figs 3 4) The latter finding is what could be expected as this organelle must be involved in the coupling between the carbohydrate component and the protein chain of the glycoprotein synthesized by—and therefore present in—the tumour cells Furthermore, the granules displayed a positive staining reaction with phosphotungstic acid (PTA) at low pH

With a modified Gomori technique, acid phosphatase activity was localized to lysosomes, certain Golgi saccules and small immature granules (Fig 5) Mature granules were always unreactive The significance of acid phosphatase activity in immature granules is not clear but it has been suggested that acid phosphatase may be involved in the concentration packaging and modification of secretory material

The overall submicroscopical features of the tumours studied were rather similar—with one exception, a case in which the patient displayed a spontaneous, preoperative facial nerve paralysis In specimens from this tumour the most striking finding was that the mitochondria were grossly abnormal in appearance Furthermore, the cells exhibited unusually smooth cell borders with only few intercellular interdigitations Lastly, in this tumour atypical junctional complexes between adjacent cells were a general feature

Our biochemical analyses revealed that the amylase content of the tumours was considerably lower than that of normal parotid gland tissue On the other hand, cyclic AMP levels were the same, or elevated, as compared with normal parotid parenchyma Previous studies have suggested an important role for cyclic AMP in controlling cell growth and proliferation, although its involvement in control of tumour growth *in vivo* is poorly understood Alterations in the cyclic nucleotide levels have been implicated in the development and progression of a variety of tumours and transformed cell lines In general, neoplastic activity has been associated with low intracellular concentrations of cyclic AMP, and in some cases with high intracellular levels of cyclic GMP The latter nucleotide has been shown to either stimulate mitosis or be present in high concentrations at sites where cells divide vigorously Malignancy, however, is not inevitably associated with low concentrations of cyclic AMP, as a number of malignant cells contain normal or even supernormal levels of this nucleotide Expanded studies on a more comprehensive material may clarify whether ultra-


1
2
3
4
5

structural studies as well as biochemical analyses of e g cyclic nucleotides may be of value for the prognostication of acinic cell carcinomas

A reference list can be obtained from the author on request

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Fig 1 Biopsy smear of acinic cell carcinoma displaying irregular clusters of monomorphous epithelial cells with pale indistinct cytoplasm ×320
Fig 2 Electron micrograph of an agranulated cuboidal tumour cell ×7900
Fig 3 Periodic acid–chromic acid silver [illegible] method Golgi complexes and cytoplasmic granules of three tumour cells are heavily stained ×10400
Fig 4 Periodic acid–bismuth subnitrate method Cytoplasmic granules of a tumour cell display a positive staining reaction ×13000
Fig 5 Acid phosphatase preparation Precipitate is demonstrated in certain portions of the Golgi apparatus ×21400

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IRRADIATION-INDUCED TUMOURS OF THE HEAD AND NECK

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Abstract Though irradiation induced tumours are uncommon they represent a well defined entity At this Hospital 14 irradiation induced head and neck tumours were encountered in 11 patients over a 10-year period The irradiation had been given for tuberculous lymph adenitis in 6 of the patients for lupus vulgaris in one, and thyrotoxicosis in another the other 3 patients had received radiotherapy for malignant tumours The interval between the treatment and the diagnosis of the tumour disease ranged from 9 to 48 years (mean 32) The induced tumours included 10 squamous cell carcinomas of the hypopharynx (4 tumours) the buccal mucosa (3) the skin (2) and the larynx (1) one poorly differentiated carcinoma of the parotid gland 2 thyroid carcinomas and 1 fibrosarcoma of the sternocleidomastoid muscle Three of the patients had multiple tumours In view of the risk of cancer—albeit a small one—associated with radiological diagnosis and radiotherapy these should be performed only on strict indications especially in young patients

The carcinogenic properties of ionizing radiation were recognized only a few years after Röntgen's discovery of X-rays in 1895 In 1902 Frieben reported the first irradiation-induced carcinoma in man—an epithelioma of the skin of the hand in a 33-year-old technician who had been testing X-ray tubes for 4 years

The biological effects of ionizing radiation, such as X-rays dermatitis, pigmentation, telangiectasies, fibrosis scarring and ulceration were described by Stevens in 1896

The application of X-rays for therapeutic purposes ultimately became widely adopted, and for many decades was considered the treatment of choice for patients with many conditions, including hyperplasia of the thymus gland, the tonsils and adenoids, tuberculous lymphadenitis in the neck haemangiomas of the head and neck juvenile laryngeal papilloma, tinea capitis acne and hirsutism Irradiation has also been given for impetigo bronchitis, menorrhagia, mastitis, and duodenal ulcer

While the oncogenic effects of radiation are well established, the actual mechanism by which the tumour is induced remains obscure Among the organs in which such tumours have been reported—apart from the skin—are the lung, thyroid gland, breast, brain, bone, soft tissue, and blood

One of the first reports of irradiation induced tumours of the head and neck was made by Lossen in 1936 Some organs and regions display a greater sensitivity to irradiation than others The tumour incidence appears not to be correlated with either the irradiation dose or the interval elapsing between irradiation and the appearance of the tumour

This paper reviews the experience of this kind of tumour in the head and neck, accumulated at this Hospital over the last decade

CASE SERIES

Over the decade from 1968 to 1977, 14 irradiation-induced tumours of the head and neck were diagnosed in 11 patients at this Hospital All had received radiotherapy for diseases of the head and neck and the irradiated field had included the tumour sites (Table I)

The benign conditions for which the radiotherapy had been administered were tuberculous lymphadenitis in the neck (6 patients aged 11–30 years), lupus vulgaris (1) and thyrotoxicosis (1), the malignant diseases occurring in a further 3 patients, aged 59–65 years, were squamous cell carcinoma of the cheek, buccal mucosa and vocal cord

Table I *Irradiation induced head and neck tumours (11 patients)*

Patient	Date of diagnosis	Sex	Primary disease	Age	Radiotherapy Dose	Radiotherapy Year	Irradiation induced tumours	Interval between radiotherapy and tumour diagnosis (years)
1 K R	1968	♀	Thyrotoxicosis	30	?	1936–37	Squamous cell carcinoma of epiglottis	31
2 A H	1969	♂	Tuberculous lymphadenitis	25	?	1924	Squamous cell carcinoma of hypopharynx	44
							Fibrosarcoma of sternocleido-mastoid muscle	48
3 F S	1971	♀	Tuberculous lymphadenitis	30	400 R	1944	Poorly diff carcinoma of parotid gland	27
4 W E	1973	♀	Squamous cell carcinoma of cheek	65	3 × 1 200 R	1964	Squamous cell carcinoma of cheek	9
5 Å B	1975	♂	*Tuberculous* lymphadenitis	27	*17 treat*ments over 3 years	1933–36	Squamous cell carcinoma of hypopharynx	42
6 K A	1975	♂	Squamous cell carcinoma of buccal mucosa	59	5 200 rad (Betatron)	1958	Poorly diff squamous cell carcinoma of buccal mucosa	17
7 A K	1975	♂	Squamous cell carcinoma of vocal cord	62	5 750 R	1959	Squamous cell carcinoma of hypopharynx	15
8 G P	1976	♂	Tuberculous lymphadenitis	13–15	?	1930–32	Papillary and follicular carcinoma of thyroid gland	43
9 H I	1976	♀	Tuberculous lymphadenitis	11	?	1922	Follicular thyroid carcinoma	37
							Follicular thyroid carcinoma	(54)[a]
							(Basal cell carcinoma)	(54)[b]
10 C T	1977	♂	Lupus vulgaris of cheek	43	?	1942	Squamous cell carcinoma of buccal mucosa (surgery followed by radiotherapy 5 300 R)	21
							Squamous cell carcinoma of buccal mucosa	35
11 E G	1977	♂	Tuberculous lymphadenitis	18	?	1932	Squamous cell carcinoma of hypopharynx (radiotherapy 6 140 R)	29
							Squamous cell carcinoma of skin (neck)	45

[a] This tumour may have been a recurrence
[b] This basal cell carcinoma was probably located outside the radiation field

For 6 of the patients (2 of them treated in the 'twenties) exact information concerning the radiotherapy was not available

The 11 patients (4 women, 7 men) ranged in age from 46 to 77 years (mean 65 years) at the time the first irradiation induced tumour was diagnosed The mean interval between radiotherapy and tumour diagnosis was about 32 years, in 1 patient 48 years had elapsed

The tumours were 10 squamous cell carcinomas (4 tumours of the hypopharynx, 3 of the buccal mucosa, 2 of the skin, and 1 of the epiglottis), 2 thyroid carcinomas (1 papillary and follicular and 1 follicular carcinoma), 1 poorly differentiated carcinoma of the parotid gland and 1 fibrosarcoma of the sternocleidomastoid muscle

Most of the patients received surgical treatment (Cases 1–3, 5, 8–11) The 2 patients with thyroid carcinoma were given postoperative radio iodine (*50* and *100* mCi of I^{131}, Cases *8* and 9, respectively) and 3 pre- or postoperative radiotherapy (Cases 3, 5 and 10) The patient with skin carcinoma of the cheek received curative radiotherapy The tumour had developed 9 years after radiotherapy for a similar carcinoma (Case 4), 4 years later there was no evidence of recurrence One of the 4 patients with hypopharyngeal carcinoma had been given curative radiotherapy, 16 years later squamous cell carcinoma of the overlying skin developed Treatment consisted in wide excision and reconstruction with a deltopectoral flap (Case 11)

Fifteen years after radiotherapy for a vocal cord carcinoma, one patient developed inoperable carcinoma of the hypopharynx (Case 7) He benefited little from palliative therapy and succumbed to the disease 6 months later

Two patients received palliative chemo therapy (Cases 6 and 10)

DISCUSSION

While the carcinogenic properties of ionizing irradiation are familiar, it is still not known whether damage to the DNA molecule (purine or pyrimidine damage or a single- or double strand break) is the cause of cell death and induction of cancer, or whether other targets such as the membrane complex are involved (Fry & Ainsworth, 1977)

Between the radiotherapy and the development of the tumour a long period usually elapses—an interval of 64 years has been reported (Martin et al , 1970)—though the latent interval may perhaps be as short as 5–11 weeks (Walter, 1950) The range for the present series was 9–48 years

The majority of the patients had received radiotherapy for tuberculosis of the cervical nodes Four male patients developed carcinoma of the hypopharyngeal mucosa, which seems to be a particularly sensitive target organ Radiotherapy for tuberculosis apparently also tends to increase the risk of malignant transformation, and likewise for lupus vulgaris (Beck, *1922*, Hornberger, *1949*) Duffy & Fitz gerald (1950) suggested that there is an aetiological relationship between irradiation and thyroid carcinoma in children More recently, the salivary glands and the breast have proved to be susceptible to the effect of radiation—a fact having important implications for breast cancer screening programs (Baral et al , 1977)

Since Beck presented the first case of post irradiation sarcoma in 1922 many irradiation induced malignant mesenchymal tumours have been reported

Multiple occurrence of irradiation induced tumours is not uncommon (Martin et al 1970) Such tumours are often not diagnosed until they are advanced, and regional and distant metastases seem to occur more often than for tumours of the same histological type having another origin than therapeutic irradiation (Hornberger, 1949)

Ionizing irradiation possesses the paradoxical dual potential of curing and inducing cancer The primary treatment for irradiation induced tumours should be surgical Though there are many doctors who do not favour the use of radiotherapy for these tumours (Martin et al , 1970), it has been used successfully in

selected cases (Petersen, 1954), the present series affords a further example

CONCLUSIONS

When the application of radiotherapy is being contemplated, due account should be taken of the possibility—albeit a small one—that cancer may be induced, and develop at some remote time

While there is no direct correlation between irradiation dose and the incidence of induced tumours it would seem that the risk varies with the dose

Any of the organs may be affected, but some of them appear to be more sensitive than others—for example, the hypopharynx

There is usually a long interval between radiotherapy and the detection of the induced tumour

Radiotherapy should be used only on the strictest indications, especially in the case of young patients

Although irradiation induced tumours are infrequent, all patients given this form of treatment should be followed up throughout life

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

Tumorbildung nach ionisierender Strahlung ist selten und tritt meistens erst viele Jahre nach vorausgegangener Bestrahlung auf Wir haben innerhalb eines zehnjährigen Zeitraums 11 Patienten mit insgesamt 14 strahleninduzierten Geschwulsten im Kopf und Halsgebiet registriert Aufgetreten sind die Tumore nach vorausgegangener Bestrahlung wegen Halslymphknotentuberkulose (6 Patienten) Lupus vulgaris (1 Patient) und Thyreotoxicose (1 Patient) Drei Patienten jedoch haben wegen eines Malignoms Strahlenbehandlung bekommen Die Latenzzeit zwischen Strahlenbehandlung und Geschwulsterscheinung betrug 9 bis 48 Jahre Die Tumore bestanden aus 10 Plattenepithelkarzinomen davon waren vier im Hypopharynx drei in der Bucca zwei in der Haut und eines im Larynx ein undifferenziertes Parotiskarzinom zwei Thyreoideakarzinome und ein Fibrosarkom im M sternocleidomastoideus Drei Patienten hatten mehr als einen strahleninduzierten Tumor Angesichts der gewiß kleinen jedoch vorhandenen malignen Entartungsgefahr sollten sowohl Strahlenbehandlung als auch röntgendiagnostische Maßnahmen vor allem bei Jugendlichen nur unter strenger Indikation vorgenommen werden

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VESTIBULAR SYMPTOMS IN MUMPS DEAFNESS

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Abstract Deafness appears in as many as 4% of adult cases of epidemic parotitis It is often severe though reversible and is usually unilateral Vertigo has been reported in mumps as have occasional cases with impaired caloric reaction Twenty cases of unilateral hearing impairment in mumps have been investigated with audiometry and electronystagmography in order to determine the degree of permanent lesions None of the patients had noted vertigo when falling ill with parotitis Five of these had normal calorics 3 were impaired and one was without response in the deaf ear No certain correlation between vertigo and permanent caloric impairment was found An interesting finding was that 5 cases without vertigo showed an impaired or absent caloric responsiveness which might confuse future diagnostics Presumably most patients with hearing impairment in mumps suffer vestibular damage but the acute vertigo in early childhood is easily overlooked No certain correlation between age at the onset of mumps and any permanent caloric disturbance was found One mumps case is described in which a severe hearing loss and caloric impairment returned to normal

Deafness due to epidemic parotitis has been known for over a century It has been thought that the hearing impairment is very uncommon, that it is unilateral, severe and irreversible (Boot 1908) Some authors have reported vestibular symptoms in connection with mumps (Beal & Naunton, 1966, Lindsay, 1959) The frequency and type of vestibular disturbance are however, not described In order to study the vestibular symptoms in cases of hearing impairment due to mumps an investigation was performed on a series of such cases

MATERIAL AND METHODS

Twenty patients with unilateral hearing impairment appearing in cases with epidemic parotitis were investigated with audiometry and electronystagmography, 9 were female 11 male The age range was 3 to 37 years the mean age being 12 years Sixteen of the patients had a totally deaf ear, 3 had a partial deafness and one had impaired hearing that returned to normal

RESULTS

Nine patients had suffered from vertigo at the onset of the hearing impairment, 11 had no history of vertigo (Table I) The mean age among the patients with vestibular symptoms was 18 years and in the non vertiginous patients 7 years The caloric test performed 2 months to 6 years after the onset of the disease showed normal responses in 11 cases and impaired responses in 9 cases There was no correlation between the history of vertigo and the outcome of the caloric test performed at this date

In one case, a nurse, 29 years of age the hearing impairment started 6 days after the onset of the parotitis and she experienced vertigo simultaneously Initially she had a 55 dB hearing loss (Fig 1), with recruitment Her caloric response was seriously impaired The hearing improved and was completely normal after 6 months Her spontaneous nystagmus disappeared after less than 1 month and her caloric response was normal after 6 months

Table I

Calorics		Vertigo	No vertigo
Normal		5	6
Impaired	partially	3	4
	totally	1	1

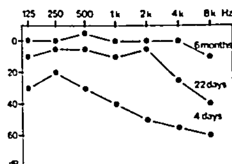


Fig 1 A case of complete hearing recovery after acute unilateral loss due to mumps

DISCUSSION

The frequency of hearing impairments in cases of mumps among 298 men in military service is reported by Vuori et al (1962) to be 4% The hearing impairment was mostly reversible and with no correlation to meningo-encephalitis These cases were all unilateral, but bilateral hearing impairment has been reported to be 20% (Everberg, 1957) Hearing impairment without obvious epidemic parotitis has been reported to be caused by parotitis virus (Dishoeck et al , 1957, Saunders & Lippy 1959) Welsh & Welsh (1963) reported that of 38 cases of sudden deafness, half of them due to epidemic parotitis, 43% had abnormal caloric reactions The virus etiology of disturbances of the facial nerve and inner ear function has been much discussed lately (Ödkvist et al 1977, Djupesland et al 1978) Lindsay et al (1959) using histopathology have shown damage in the organ of Corti and stria vascularis in deafness due to measles Many of the hearing impairment cases caused by virus infection are reversible which indicates that the damaged parts are well able to regenerate, obviously true also for the vestibular part of the inner ear, as some of our vertiginous patients achieved a normal caloric response The percentage of impaired caloric responses among our 20 patients (45%) seems to tally with reports on sudden deafness of mixed origin (Welsh & Welsh, 1963) One important observation is that 5 patients who had not noticed any vertigo at a later date showed an impaired caloric response These cases could subsequently have become objects for tumour investigation if the connection between the epidemic parotitis and the inner ear lesion had not been known The fact that a vestibular lesion can appear without any obvious symptoms might be explained by the young age of the patients and the consequent quick adaptation of the vestibular impairment Furthermore a spontaneous nystagmus may easily be overlooked in a child in bed running a high temperature Our case with total restitution of hearing and vestibular function indicates that some cases may elude diagnosis

The complications of epidemic parotitis have caused some authors (Bjorvatn & Sköldenberg, 1978) to recommend mumps vaccination

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FIBRINOLYTIC ACTIVITY IN PATIENTS WITH SUDDEN SENSORINEURAL HEARING LOSS

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Abstract Fibrinolytic activity and capacity were studied in a group of 18 patients with sudden sensorineural hearing loss of unknown etiology The fibrinolytic activity and capacity were found reduced in 12 patients No distinct changes in platelet aggregation in vitro could be demonstrated Further repeated studies in this category of patients should be performed

Cases of sudden sensorineural hearing loss are normally divided into those with and those without known etiology In the first group are included cases due to ototoxic drugs, temporal bone fracture, inner ear concussion, surgical injury, acusticus neurinoma, multiple sclerosis, etc

According to Fowler (1950) and Mattox & Simmons (1977), cases of unknown etiology [illegible] the majority group

The expression "sudden" is understood dif[illegible] by different otologists In most cases [illegible]ive loss of hearing develops quickly within a few minutes Other patients will wake up in the morning realizing the loss of the hearing Most seldom the loss of hearing develops during hours or days

The aim of the present work has been to study the fibrinolytic activity and platelet aggregation in vitro in patients with sudden sensorineural hearing loss making an analogy to other vascular episodes such as transitory cerebral ischemia (Andersen & Gormsen, 1976) or completed stroke (Lou et al 1977)

MATERIALS AND METHODS

The material consists of 18 patients 10 men and 8 women with an average age of 38 4 years (17–61 years) It was of decisive importance that the patient could define the episode subjectively as a sudden loss of hearing and that the audiometric examination showed a perceptive hearing loss The time period between the acute symptoms and the first otologic examination was 0–16 weeks, with an average of 3 weeks The observation period was up to 90 weeks, averaging 20 weeks The patients went through a general physical examination The otological examination included tone audiometry, speech audiometry, tone decay, examinations of stapedius reflexes *ad modum* Metz and a vestibular examination with a calorigram X-rays of internal pori acustici were performed None of the patients suffered from diabetes mellitus, lues, thyreotoxicosis blood diseases or hypertension Five patients suffered from hypertriglyceridemia All cases were unilateral The vestibular function tests followed normal patterns

Fibrinolyses

The fibrinolytic activity was studied as described elsewhere (Andersern & Gormsen, 1976) The spontaneous lysis time of the plasma euglobin fraction is in normal persons with our methods, shorter than 180 min and the lysis time after venous compression for 5 min to a blood pressure between the systolic and the diastolic is shorter than 60 min (fibrinolytic capacity)

Platelet aggregation

The platelet aggregation in vitro was studied as described elsewhere (Gormsen, Nielsen &

Table I *Fibrinolytic activity of plasma e gl bul n fraction*

Lysis time	Spontaneous lysis time (normal <3 h)	After venous compression (normal <1 h)
>24 h	7*	5*
3–24 h	5	1
1 3 h	6	3
<1 h	0	9
Total	18	18

* Five of these pts had hypertriglyceridaem a

Andersen 1977 Petersen & Gormsen 197 in a Payton aggregometer estimating tl threshold concentration of ADP (adenosinı phosphate Sigma Chemical Co St Lou USA) and epinephrine (Gauche Rhone Po lence Paris) ADP was used in a final conce tration of 0 25–0 50–1 0–2 0 and 5 0 μM aı epinephrine in a final concentration of 0 0 0 1 and 1 μg per ml

RESULTS

Fibrinolytic activity

The results are given in Table I Both tl spontaneous and the fibrinolytic capacity we significantly decreased in the group of patien in question 12 having significantly reduce fibrinolytic activity according to our method In 5 of these an elevation of the triglycerid in the blood was found

Platelet aggregation

The results are shown in Table II In most the patients the aggregation parameters are d fined by threshold concentrations of ADP ar epinephrine and were within the normal rang Only in 2 was a lower threshold found illu trating hyperaggregability in vitro and showed a decreased aggregability Wheth this was due to drugs is unknown

DISCUSSION

In 12 of 18 patients suffering from sudden se sorineural hearing loss without any oth

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EAR DAMAGE DUE TO DIVING

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Abstract Diving injury may affect all parts of the ear during all types of diving Divers are regularly exposed to noise and 120 dB(A) is measured during ventilation of a hyperbaric chamber Ear canal squeeze possibly with drum perforation may give a transient conductive hearing loss Middle ear squeeze possibly with drum perforation also gives a transient conductive loss Inner ear barotrauma possibly with perilymph fistula most often results in transient vertigo and lasting sensorineural high tone loss often resembling a noise induced loss Decompression sickness and gas embolism can also damage the inner ear A change of breathing gas during deep diving has damaged the labyrinth most likely due to counter diffusion The authors have seen two cases of oval window perilymph fistula resulting from diving One of them also suffered a burst ear drum on the same side His hearing returned to normal after surgical repair

Noise

In a diver s working environment there is much noise The flow of breathing gas through a standard helmet may produce 112 dB(A) and ventilation in a hyperbaric chamber, 120 dB(A) (Farmer, 1977) Thus divers run a grave risk of developing noise induced injury

Barotrauma

Usually the damage resulting from the varying hydrostatic pressure is recognized as the typical injury of divers In water, the pressure rises with 1 atmosphere per 10 metres depth and the gas volume changes in inverse proportion to the pressure

(a) *Barotrauma of the ear canal* If the ear canal is closed off, a relative underpressure will develop during descent The ear drum will bulge outward and the blood vessels in the ear canal and ear drum will be dilated Edema is produced and subepithelial bleeding may occur resulting in hemorrhagic bullae in the skin and on [illegible] causing bleeding from the ear The ear drum may also burst outwards (Edmonds, 1973) The otoscopical findings are dark blisters and possibly bleeding which may hinder survey of the ear drum To decide whether the drum is perforated one may listen for perforation sound during Valsalva's or Politzer's manoeuvres, or see how the drum moves, when using a pneumatic otoscope A possible hearing loss will be of the conductive type and the condition is called *barotrauma meatus acusticus externus barotitis externa* or *ear canal squeeze* Prophylactically it is effective to fill the ear canal with water prior to the dive or place the edge of the face mask under the edge of the hood to let compressed gas into the hood during the dive Usually no therapy is necessary

(b) *Barotrauma of the middle ear* If the Eustachian tube does not function well the middle ear will become a closed space where changes equal to those described above may occur The ear drum will bulge inward, the mucosal lining becomes injected and edematous with transsudation to the middle ear cavity Bleeding may occur and the ear drum may implode Otoscopy may reveal fluid in the middle ear, possibly hematotympanon and sometimes perforated ear drum (Edmonds, 1973) This condition is called *barotrauma auris media, barotitis media* or *middle ear squeeze* To avoid the condition people with poor Eustachian tube function should not dive Further one should strongly discourage diving during upper respiratory tract infection Whenever problems with pressure equilibration to the ears occur during diving the dive [illegible] interrupted Usually no therapy is

necessary The transient hearing loss is of the conductive type

(c) *Inner ear barotrauma with membrane rupture and/or bleeding* If pressure equilibration to the middle ear is difficult the diver will perform Valsalva's manoeuvre This elevates the central venous and cerebrospinal fluid pressure, which propagates through the perilymphatic duct to the inner ear where it may cause bleeding and a sensorineural hearing loss (Goodhill 1971) This condition is called *barotrauma auris internae* and will usually need no therapy other than rest in bed The patient should avoid anything which may raise the central venous and cerebrospinal fluid pressure If the hearing does not normalize the diver should consider to stop diving

(d) *Inner ear barotrauma with perilymph fistula* If the diver in spite of doing Valsalva's manoeuvre does not succeed in equilibrating the middle ear pressure, there will simultaneously with the raised inner ear pressure still exist a relative underpressure in the middle ear This pressure difference between the inner and the middle ear may lead to rupture of the round window membrane and/or the annular ligament (Goodhill 1971) Common symptoms from a *fistula perilymphatica barotraumatica* are vertigo and/or sensorineural hearing loss If a fistula is suspected tympanotomia will be necessary to establish the diagnosis and close any fistula The prognosis is usually good as far as social hearing is concerned but a permanent sensorineural high tone loss is common Prophylactically a diver should stop a dive whenever he has difficulty in clearing his ears rather than to try forced Valsalva's manoeuvres

(e) *Decompression sickness* In this condition, gas bubbles can damage the labyrinth and cause vertigo and sensorineural hearing loss (Edmonds 1973) Recompression and hyperbaric oxygen is the logical and correct treatment if this diagnosis is possible according to diving tables

(f) *Gas embolism* If a SCUBA diver has to perform a free ascent and the expanding breathing gas is not allowed to escape freely from his lungs pulmonary alveolae may burst and allow compressed gas to enter the blood stream This can then damage the labyrinth but in such cases the ear symptoms will usually be overshadowed by life threatening symptoms The therapy is prompt recompression and hyperbaric oxygen

Counter diffusion

During experimental deep dives with heliox damage to the labyrinth has occurred in connection with a change of breathing gas even at stable depth The mechanism may be damage to the inner ear membranes from bubbles arising due to counter diffusion (Farmer, 1977) Since no effective therapy is known any such gas shift should be avoided

Cases

Several cases of hearing loss resulting from diving have been reported from Norway (Molvær et al , 1978) and in our ward we have operated on two divers with oval window fistula One of them was a skin diver who dived to the bottom of a 4 metre deep swimming pool (Gundersen et al , 1978) and the other a SCUBA trainee who dived to 7 metres in the sea He also got an ear drum perforation on the same side (Molvær et al) Both the fistula and the drum perforation were closed surgically and postoperatively his hearing normalized

Audiology

The hearing losses resulting from inner ear damage due to diving are usually localized in the high tone range and may look like a noise-induced loss (Molvær et al 1978) A history and audiogram from prior to the damage are usually necessary to establish the etiological diagnosis

ZUSAMMENFASSUNG

Samtliche Teile des Ohres können durch alle Arten von [illegible] Taucher sind regelmäßig [illegible]

[illegible]

übergehenden mechanischen Hörverlust geben [illegible]

media eventuell mit Perforation des Trommelfelles gibt auch einen vorubergehenden mechanischen Hörverlust Barotrauma auris interna eventuell mit Fistula perilymphatica resultiert gewöhnlicherweise in vorubergehendem Vertigo und dauernd neurogenem Hörverlust in den Diskant Tonen so daß es manchmal einem gerauschindu zierten Horverlust ähnlich sein kann Dekompressions krankheit und Alveolenruptur mit Gasembolien können auch das innere Ohr schaden Wechsel von Atemgas wahrend Tieftauchen hat auch das Labyrinth geschadet meist wahrscheinlich wegen eines Gegendiffusionsphanomen Die Verfasser haben zwei Fälle von Fistula peri lymphatica in Fenestra vestibuli von Tauchen verur sacht operiert Einer davon hatte auch perforierter Trom melfell auf derselben Seite Sein Gehör hat sich nach chirurgischer Behandlung normalisiert

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IMPEDANCE SCREENING FOR MIDDLE EAR DISEASE

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Abstract Pure tone audiometry has hitherto been employed as the sole means of indentifying ear pathology During the last decade it has been demonstrated that even hearing which is normal according to pure tone sweep check screening audiometry (20 dB) can be associated with middle ear pathology With the intention of identifying cochlear as well as middle ear pathology, a study was initiated in which pure tone testing was combined with impedance measurements as a screening test The intention has been to determine a screening level at which pathology is identified and the number of false positive is minimized A combination of tympanometry (≤ -150 mmH_2O) and pure tone screening at 0 5 and 3 kHz is recommended as a screening procedure for the identification of cochlear as well as middle ear pathology

Since it was recognized that pure tone screening testing was inadequate for the identification of middle ear pathology (Brooks, 1969) great efforts have been made to improve the screening procedure The goal must be a screening method which identifies cochlear as well as middle ear pathology With such intentions, we started a project in Goteborg in 1972 in which a combination of impedance measurements and pure tone test has been employed

METHOD AND MATERIAL

In the first study the material consisted of 10-year-olds and in the following three studies of 7-year old children from Goteborg Tympanometry, stapedius reflex testing and pure tone sweep check testing have been used A Madsen impedance bridge (probe frequency 2 2 kHz) with contralateral stimulation has been used in all but the last study in which a Madsen impedance bridge with ipsilateral stimulation has been imployed Different borderline values for pathology have been employed in the various studies (Table I)

RESULTS

Our first study (Renvall et al , 1973) revealed a high percentage pathologic values using impedance audiometry in comparison with otoscopy and pure tone screening (Table II) This encouraged us to expand the study as well as to alter the screening level The results of the second study (Renvall et al , 1975) demonstrated pathologic pure tone screening in 6 5%, pathologic tympanometry in 13 5% and abnormal stapedius reflex test results in 32% Analysis of the distribution of the middle ear pressure showed that 10% of the ears with no elicitable reflex using contralateral stimulation had middle ear pressure value between ±50 mmH_2O Therefore in order to reduce the number of false-positive responses we decided to revise our criteria yet again Since a conductive component of hearing loss can vary between the ears of one and the same individual, the stapedius reflex threshold ought to be measured by ipsilateral stimulation Such equipment was not available at the time of our next study, so stapedius reflex testing was excluded (Table II) This study (Lidén & Renvall, in press) demonstrated that 10% of the ears tested had middle ear pressures ≤ -150 mmH_2O and that 25% of the ears with flat tympanograms had normal pure tone test results In order to eliminate ears with occasional episodes of subnormal middle ear pressure from being identified by our screening test we introduced a repeat test 4 weeks later accord

Table I *Borderline value used in four different studies between 1972 and 1977*

Study no	Screening audiometry	Tympanometry (mmH_2O)	Stapedius reflex (db HL)
I	>20 dB HL on 2 or more frequencies	≤−80	>95
II	>20 dB HL on 2 or more frequencies	≤−100	>95
III	>20 dB HL on 2 or more frequencies	≤−150	–
IV	>20 dB HL at 0 5 or 3 kHz	≤−150	≥100

Table II *Results from four different studies in which a combination of impedance audiometry and pure tone test has been used*

Study no	Screening audiometry (%)	Otologic examination (%)	Tympanometry (%)	Stapedius reflex (%)
I	1.5	8	12	35
II	6 5	–	13 5	32
III	–	–	10	–
IV	4 6	–	9 8	28 6

ing to Brooks (1976) With this approach we revised our screening program in 1977 as shown in our fourth study (Table II) Ears which failed the repeat test were referred to an oto audiological department for a final test and ears now failing were inspected by an oto laryngologist By this repeated testing technique we reduced the failure rate from 27% at the first test and 12 5% at the second to 9 6% at the final test

Our results from the fourth study demonstrate that ears in which no reflex could be elicited at contralateral stimulation (110 dB HL) had a middle ear pressure of ±50 mmH_2O in 14 5% and ears in which no reflex could be elicited at ipsilateral stimulation (110 dB HL) had middle ear pressure of ±50 mmH_2O in 17% It can thus be concluded that the use of ipsilateral stimulation to elicit the stapedius reflex does not eliminate false positives that is ears with normal middle ear pressure and pathologic stapedius reflex test

CONCLUSION

A combination of tympanometry and pure tone screening is recommended as the best screening procedure for identification of cochlear as well as middle ear pathology A repeat test procedure is recommended The screening level recommended is a middle ear pressure of ≤−150 mmH_2O and pure tone screening 20 dB at 0 5 and 3 kHz

ZUSAMMENFASSUNG

Pure Tone Audiometrie ist bis heute das einzige Mittel welches eine Identifikation der Pathologie der Ohren ermoglicht Im letzten Dezennium hat es sich gezeigt daß normales Gehor nach einem Pure Tone sweep check Test mit pathologischen Veranderungen des Mittelohres vereint sein kann Mit der Absicht der Identifikation sowohl der cochlearen als der Mittelohrpathologie ist eine Studie ausgearbeitet worden bei welcher man den Pure Tone Test mit der Impedance Methode kombiniert hat Das Screenings Niveau für die Tympanometrie und des Stapediusreflextestes ist wahrend der Studie verändert worden mit dem Ziel ein Screenings Niveau fest zustellen bei welchem die pathologische Identifikation nach falsch positiv minimum ist Erfahrungsgemaß ist eine Zusammenstellung der Resultate der Tympanometrie (≤−150 mmH_2O) und der Pure Tone Screening (0 5 und 3 kHz) eine Screeningsmethode welche für die Identifikation von sowohl der cochlearen als auch der Mittelohr Pathologie empfohlen wird

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ZUSAMMENFASSUNG

212 von 242 Patienten (88%), die an chronischer seroser Otitis media im Jahre 1972 litten, wurden fünf Jahre später neu untersucht. Insgesamt 668 operative Eingriffe sind gemacht worden, 99 Patienten wurden nur einmal operiert, wahren ein Patient 12 Operationen durchmachen mußte. Das Audiogramm was besser als 20 dB in 88% der Ohren bei der Untersuchung 1977, wahrend nur 57% einen Typ A Tympanogramm hatten. Normale Otoskopie wurde in 40,5% der Ohren gefunden, bestehende sekretorische Otitis media in 11%, atelektatisches Ohr in 2,8%, und chronische suppurative Otitis media in 2,6%, während die restierenden Ohren 42,9%. Narben unterschiedlichen Grades und Myringosklerosis zeigten.

A list of literature references may be obtained from the authors on request.

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THE EFFECT OF ADENOIDECTOMY ON SECRETORY OTITIS MEDIA IN CHILDREN

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Abstract 139 patients 80 boys and 59 girls aged 1½–13½ years suffering from serous otitis media based on otoscopy and tympanometry had adenoidectomy performed and no other surgical measures taken The efficiency of the treatment was assessed 2–4 months later Initially flat tympanometry curves were found in 155 ears negative middle ear pressure in 70 ears and low curves in 20 ears 78% of all ears and 73% of the ears with flat curves were seen to normalize during the observation period

In children of pre-school age the incidence of secretory otitis media has reached almost epidemical numbers over recent years Several causes may be pointed out (1) advanced diagnostics, particularly the use of tympanometry, (2) greater effort to trace and treat hearing loss in pre school children, (3) possibly a genuine increase in the number of cases of secretory otitis media

The most common treatment for secretory otitis media is adenoidectomy in combination with simultaneous insertion of tympanostomy tubes, leading to an improvement in hearing as long as the tube is in situ

The object of this study is to investigate if adenoidectomy alone subsequently supplemented by conservative treatment as Politzer and nasal decongestants are sufficient in the treatment of secretory otitis media since this kind of treatment proved effective in many cases before 1960

EQUIPMENT

The apparatus employed was a Madsen Impedance meter (type ZO 72) connected to an X-Y writer Negative middle ear pressure ≤ -100 mmH$_2$O, flat curves or low curves with compliance ≥ 6 on the relative compliance scale but with a minimum of impedance within $+/-100$ mmH$_2$O were considered abnormal

SUBJECTS

The material consists of children from Copenhagen City admitted to hospital over a period of 1½ years due to adenoids demanding surgical treatment and secretory otitis media, the latter diagnosis being established by otoscopy and tympanometry In children more than 5 years of age an average hearing loss of more than 10 dB testing three frequencies was considered pathological Children previously treated with adeno tonsillectomy or insertion of tympanostomy tubes were not included in this material When adenoidectomy had been performed the patients were clinically assessed 2 months later If secretory otitis media persisted, treatment with nasal decongestants and politzeration was given and instruction in the Valsalva manoeuvre likewise If secretory otitis media still persisted 3 months after the initial therapy was given the patient was readmitted to the hospital and re-adenoidectomy and in some cases insertion of a tympanostomytube was carried out

The study comprised a total of 139 patients, 80 boys and 59 girls aged 1½–13½ years (mean 5 years) all showing abnormal otoscopy and

ears, i e 155

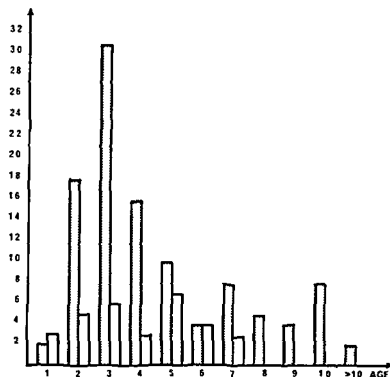


Fig 1 The effect of adenoidectomy in relation to age Successfully treated 108 patients (▨) Unsuccessfully treated 31 patients (□)

curves, 70 with negative pressure and 20 with low curves

RESULTS

Audiometry was carried out in a total of 107 ears, being normal in 15, showing conductive hearing loss of 10 dB–<20 dB in 32, 20 dB–<40 dB in 57 and ≥40 dB in 3 ears including 1 perceptive hearing loss The effect of the treatment is shown in Table I A few patients did not present themselves at the pre-set clinical control 2 months after the treatment and were resummoned for consultation 6 months later 78% of all the ears were normalized during the observation period 73% of the ears with flat curves were normalized over the same period of time and 87% of the ears with negative pressure and low curves as well, this difference shows statistic significance (χ^2 test) Table II shows the supplementary treatment administered when adenoidectomy failed

Fig 1 shows the relationship between age and postoperative results

Table I *The effect of adenoidectomy on secretory otitis media evaluated by tympanometry*

		Post-operatively			
		2 months		3–6 months	
Pre-operatively		Normal	Not normal	Normal	Not normal
Low	20	6	14	8	6
100<200	19	13	6	5	1
≥200	51	20	31	26	5
Flat	155	53	102	60	42
Total	245	92	153	99	54

Table II *Treatment of 54 ears suffering from persisting secretory otitis media after initial adenoidectomy*

22	Re adenoidectomy + insertion of tympanostomy tubes
8	Re-adenoidectomy + large adenoids
[illegible]	[illegible]
	[illegible] planned
1	Regarded as successfully treated at negative pressure = −150 mmH₂O

DISCUSSION

78% of patients suffering from adenoids with flat curves or negative pressure were successfully treated by adenoidectomy The value of the insertion of a tympanostomy tube, however, is indisputable due to the improvement in the hearing gained, though not quite without risk, as pointed out by Eskar (1977) stating that rare sequelae like persisting defective eardrum, permanent retraction and cholesteatoma have been seen The risk of infection in about 15% of all cases is the most frequent complication In most cases the hearing loss has been acceptably small as a reduction of more than 40 dB was found only in 3 ears (2 patients), one of which had cochlear damage

In a pilot study carried out prior to this present study, adenoidectomy and simultaneous myringotomy with aspiration of glue, compared with adenoidectomy alone, showed no difference in efficiency, and consequently the first type of treatment mentioned has not been assessed in this study Prevailing failure of therapy in the older age groups might be expected, partly because of the natural reduction in size of the adenoids at the beginning of school age and partly because longer-lasting symptomatology might be expected Verification of this has not been possible in this study

A method useful in selecting patients for primary insertion of a tympanostomy tube still remains to be elaborated

ZUSAMMENFASSUNG

Bei 139 mittels Otoskopie und Tympanometrie diagnostizierten Otitis media Patienten 80 Jungen 59 Mädchen im Alter von 1½ bis 13½ Jahren wurde als einziger chirurgischer Eingriff Adenotomie durchgeführt Präoperativ wurden in 155 Mittelohren flache Tympanometrie Kurven in 70 negativer Mittelohrdruck und in 20 Mittelohren niedrige Kurven ermittelt Der Behandlungseffekt wurde nach 2 bis 4 Monaten kontrolliert Am Ende der Observationsperiode wurde Normalisierung der gemessenen Parameter in 78% aller untersuchten Mittelohren gefunden während nur in 73% der Fälle, die initial flache Kurven aufzeigten Normalisierung eintrat

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MIDDLE EAR MUCOSA IN CLEFT PALATE CHILDREN

A Scanning Electron Microscopic Study

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Abstract Twenty three promontory mucosal specimens from cleft palate children were studied by scanning electron microscope Comparative studies were made using light and transmission electron microscopy The mucosal lining was pseudostratified columnar with microvilli a few ciliated cells with secretory granules and numerous goblet cells A few mucous glands could be seen in certain areas Microvilli with bulging tips and cilia with microknobs were common phenomena The submucosa were inflamed with leukocytes macrophages plasma cells and lymphocytes indicating the presence of both cellular and humeral defence mechanisms

Most children with cleft palate have from birth onward a serous or secretory otitis media (SOM) The main reason is, it seems, dysfunction of the Eustachian tube due to muscle incompetence Periodically the hearing can be affected, but the long term hearing results are favourable (Møller, 1975)

In order to study the middle ear mucosa in cleft palate children, biopsies were taken from the promontory area during myringotomy and observed with a scanning electron microscope (SEM) Our results were compared with the findings reported by Lim (1976), Shimada et al (1972) and Hentzer (1970), describing the normal epithelium to be simple squamous, cuboidal or columnar with microvilli and ciliated cells on the promontory, indicating that even normal epithelium can differ considerably

MATERIAL AND METHODS

Twenty three cleft palate patients underwent bilateral myringotomy with general anesthesia Biopsies were taken from the lower part of the promontory and specimens were processed for scanning electron microscopy The material consisted of 18 patients (under 2 years) with bilaterally mucoid effusions, 2 patients (2–4 years) with bilaterally mucoid effusions and 1 patient (2 years) with bilaterally serous effusions One patient (2 months) had bilaterally dry ears and 3 patients (8–10 years) had dry ears the last 2–4 weeks prior to myringotomy

In order to remove cellular debris from the surface of the specimens, the material was briefly rinsed with physiological saline prior to fixation with 2% glutaraldehyde and 1% OsO_4 Both fixatives were made up in 0 1 M cacodylate buffer at pH 7 2 and kept ice cold during fixation The tissue was dehydrated in increasing concentrations of ethanol, transferred to acetone and critical point dried using CO_2 After coating the specimens with a thin film of gold, using the diode-sputter technique, they were examined in a Philips (PSEM 500) scanning electron microscope operated at 20 kV

Specimens which had been studied in the SEM were embedded in Epon 812 After polymerization the blocks were cut with glass knives on a Reichert ultramicrotome Thick sections (1–2 μm) were stained with Toluidine blue for observation in the light microscope, while ultrathin sections were stained with uranyl acetate and lead citrate according to standard techniques for correlative studies in the transmission electron microscope (TEM)

In addition biopsy samples from 3 cleft palate children (2, 7 and 11 years of age) with mucoid effusions were fixed as described

above and directly processed for TEM investigation in a Philips 300 electron microscope operated at 80 kV

RESULTS

With one exeption, all of the specimens were covered in part with a mucous blanket This was even true in the clinically dry ears Light *microscopy revealed that the epithelium* was pseudostratified columnar and the apical surface of most cells was covered with numerous microvilli Occasional ciliated cells were also noted (Fig 1)

A 2 year-old girl had the soft palate closed 6 days prior to myringotomy She experienced serous fluid in the middle ears for a few postoperative weeks before the ears became air-filled again The epithelium in this case was found to be flat (Fig 2) No sign of inflammation was found in the submucosa

Secretory doplets could be seen, typical of goblet cell secretion as described by Tos et al (1975) and Lim (1976) Bulging of the microvilli was apparent in some areas (Fig 3) We found both goblet cells and epithelial cells—as well as ciliated cells—to have microvilli at the surface

Some of the ciliated cells had large numbers of microknobs on the ciliar surface (Fig 4) These structures were mainly located at the tip and upper part of the cilia

Strands from mucous glands were observed *in a few places The presence of such glands*, as has been described by Tos et al (1973), was confirmed by our correlative light microscopy and TEM studies

The submucosa were dominated by numerous large capillaries A striking feature was the large quantities of inflammatory cells (Fig 5) In most specimens leukocytes and lymphocytes dominated the picture, along with plasma cells and macrophages

DISCUSSION

The promontory mucosa in cleft palate patients with SOM is considerably thickened, with a pseudostratified columnar epithelium and with numerous inflammatory cells in the submucosa A considerable proportion of the epithelial cells are goblet cells and a few mucous glands could be seen in our specimens The microvilli in some areas revealed considerable bulging of their tips, which may be the morphological manifestation of secretory activity and/or fluid exchange In some ciliated cells with a large number of microknobs covering the ciliar surface, TEM studies showed that these structures contained an electron dense material and that the wall was an extension of the ciliar membrane A few other knobs ballooned from the cilia and resembled more the kind described by Dahlgren et al (1972) and others (Mecklenburg et al, 1973, Baldetop et al, 1977) Support for the *view that the microknobs represent a local* secretory activity is found in the appearance of numerous vesicles in the apical cytoplasm of the ciliated cells, as was also reported by Hentzer (1972)

However, until the true nature of the microknobs is elucidated, the possibility remains that they may be degenerative phenomena

The number of atypical cilia seems to be high in the present material The majority of cilia had a normal 9+2 pattern, but compound cilia were frequently observed Such cilia have previously been found in the middle ear mucosa by Kawabata et al (1969)

One patient had transient serous fluid in the middle ear, due to impaired Eustachian tube function alone In this patient the promontory epithelium was flat and no secretory capacity could be demonstrated The serous fluid was most likely a transudate, as described by Gundersen et al (1971)

Interestingly enough, a 2 month old girl had air in both middle ears, but our microscopical studies showed a high mucosal lining with a picture indistinguishable from the children with mucous filled middle ears Within a few months, during which no acute infection occurred (to our knowledge), the girl developed secretory otitis media in both ears

S

C

1

2

[illegible]

(*C*) and the mucous blanket (*S*) can be seen

Fig 2 SEM, ×11 700 Flat epithelium on the promontory in a cleft palate child with serous otitis media lasting a few weeks due to soft palate closure



Fig 3 SEM ×11700 Promontory surface Numerous microvilli (*M*) with t p bulging a cil ated cell (*C*) and secretory granule (*S*)

Fig 4 SEM ×11700 Cilia (*C*) with microknobs

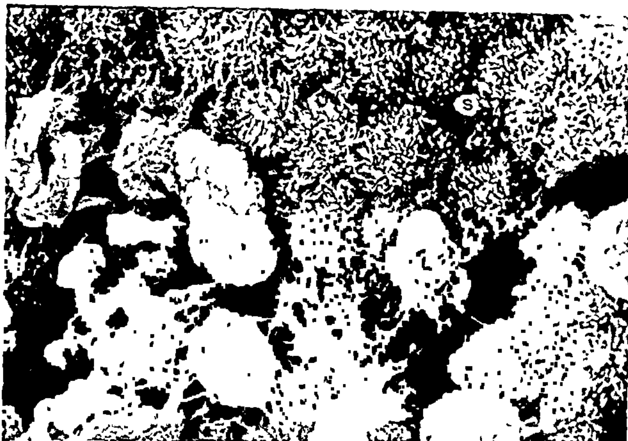


Fig 5 SEM ×2925 Submucosa near the mucosal lining Inflammatory cells (*L*) can be seen Microvilli (*M*) and secretory granule (*S*) on the surface

It is important to note that patients selected for this material had no history of acute otitis media in the previous months and no clinical signs of acute infection at the time of myringotomy Nevertheless all promontory specimens (except the case of the patient with transient serous fluid) showed a marked inflammatory response indicating both cellular and humoral defence systems to be intact The high secretory capacity of the middle ear epithelium in our cases is associated with inflammation in the thickened submucosal layer

ZUSAMMENFASSUNG

Eine Studie uber die promontonale Schleimhaut bei Kindern mit Gaumenspalte 23 Praparate der Promontonalschleimhaut wurden mit einem Scanningelektronenmikroskop untersucht Vergleichbare Studien wurden transmissionsmikroskopisch und lichtmikroskopisch durchgefuhrt Die Schleimhaut zeigte ein hohes Zylinderepithel mit Mikrovilli und wenigen Zilien Es wurden sekretorische Tropfchen von Becherzellen gefunden und auch einige Schleimhautdrüsen Die Mikrovilli waren bulging und die Zilien mit Mikroknobs" bedeckt In der Submucosa wurden Zeichen einer chronischen Entzundung mit Granulozyten Plasmazellen Lymphozyten und Makrophagen gefunden

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TONSILLECTOMY AND IMMUNE RESPONSES

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Abstract Immune responses were examined in 10-year old boys just before and one month after tonsillectomy and in young male adults tonsillectomized 10 years earlier All the parameters studied were found to be quite normal

Many infectious agents gain access to the human body through the mucosa of nasopharynx and respiratory tract The nasopharyngeal mucosa, tonsils and adenoids have therefore important anatomical location and these tissues are inconstant contact with the external environment, and constitute the primary sites of initial exposure to inhaled or ingested antigens

The tonsils are immunologically reactive lymphoid tissues, which manifest specific antibody, and B- and T-cell activity in response to a variety of antigens Many studies have demonstrated that tonsillar tissue can synthesize various types of immunoglobulins and specific antibodies, and of the lymphoid tissues the tonsils contain the largest number of IgE-producing cells (Gitlin & Sasaki, 1969, Sloyer et al , 1973, Morag & Ogra, 1975)

Removal of tonsils and adenoids has been shown to decrease the antibody response to polio viruses (Ogra, 1971) It has also been suggested that the incidence of Hodgkin's disease may be higher among tonsillectomized persons (Vianna et al 1971), but there are also studies where the results cannot support this findings (Ruuskanen et al , 1971, Johnson & Johnson, 1972)

MATERIAL AND METHODS

The material consisted of two groups of male patients The first group comprised eight 10-year-old boyd who were tonsillectomized due to recurrent tonsillitis The following studies were done just before and one month after tonsillectomy counting of leukocytes, lymphocytes and E-rosette-forming cells (T-cells), lymphocyte responses to phytohaemagglutinin M (PHA), concanavalin A (ConA) and purified protein derivative of tuberculin (PPD) and serum immunoglobulins A, G and M

The other group consisted of 9 young male adults (mean age 20 years), who had been tonsillectomized about 10 years earlier In addition to the studies done in the first group the following immunological parameters were measured NBT test, C3, C4 and lymphocyte responses to tetanus toxoid and antibodies to polio, dephtheria, tetanus and pertussis

Our routine method for lymphocyte transformation using whole blood was used (Eskola et al , 1976) Antibodies to tetanus, diphtheria and pertussis were measured using the ELISA technique (Viljanen & Ruuskanen, to be published)

RESULTS AND DISCUSSION

Tonsillectomy had no immediate effect on total leukocyte, lymphocyte or T lymphocyte counts or on the immunoglobulin A, G and M levels, which were also normal preoperatively (Table I) There are reports of decreased IgA levels in patients undergoing tonsillectomy (Donovan & Soothill, 1973, Östergaard, 1976) However, the patients with low IgA levels in those reports were younger than in our material where the patients were 10 years old Furthermore, lymphocyte responses against

Table I *Leukocyte, total lymphocyte and T-lymphocyte counts, and immunoblobulin A G and M levels just before and one month after tonsillectomy (8 patients)*

	Before tonsillectomy (mean ± S D)	After tonsillectomy (mean ± S D)
Total leukocytes ×10⁶/l	7 298±2 400	6 375±2 275
Lymphocytes ×10⁶/l	2 906±498	3 117+932
T lymphocytes ×10⁶/l	1 710±339	2 009±560
IgA g/l	1 1±0 4	1 1+0 4
IgG g/l	10 1±1 9	9 5+2 1
IgM g/l	1 4±0 5	1 4±0 5

mitogen and PPD stimulation were not affected by tonsillectomy (Table II) The responses both before and after operation did not differ from the normal values of our laboratory

The total leukocyte counts and all the immunological parameters studied were within normal values in those young male adults who had been tonsillectomized 10 years earlier (Tables III and IV) Even the antibody-forming capacity against different polio virus types was very good Ogra (1971) has found a diminished local antibody response against oral poliovaccine as long as 7 months after operation We did not study the local antibody formation after tonsillectomy but 10 years after tonsillectomy the antibody titres against polio viruses in the serum were quite normal This finding may indicate that these persons however already have sufficient protection against polio

Table II *Lymphocyte transformation before and one month after tonsillectomy (8 patients)*

Stimulant (µg/ml)		Before tonsillectomy (cpm ± S D)	After tonsillectomy (cpm ± S D)
PHA	500	44 949± 9 102	37 832± 7 459
	100	42 654+14 858	30 183±14 996
	20	3 987+ 6 160	4 905± 2 178
Con A	100	42 879+ 9 090	36 905± 5 221
	20	25 889± 9 396	16 849± 5 523
	4	7 887± 4 706	14 591± 3 976
PPD	100	909± 1 274	399± 442
	1	1 269+ 1 756	354± 175

Table III *Leukocyte, total lymphocyte and T-lymphocyte counts immunoglobulin A, E, G and M C3 and C4 levels, NBT results, antibodies to diphtheria, tetanus, pertussis and polio in young male adults (9 patients, mean age 20 years) tonsillectomized 10 years earlier*

	Tonsillectomized persons (mean ± S D)	Normal range
Total leukocytes ×10⁶/l	6 450±2 720	3 000–10 000
Lymphocytes ×10⁶/l	2 163±523	600–4 500
IgA g/l	1 8±0 5	0 3–2 3
IgE U/ml	57 2±74 4	0–150
IgG g/l	10 1±1 0	7 0–15 0
IgM g/l	1 7±0 4	0 5–2 1
C3 g/l	0 96±0 17	0 6–1 5
C4 g/l	0 18±0 08	0 12–0 34
NBT %	11 3±8 4	
Diphtheria antibodies*	50±24	
Tetanus antibodies*	62±37	
Pertussis antibodies*	111±18	
Polio 1 antibodies•	235±242	
Polio 2 antibodies•	597±391	
Polio 3 antibodies•	789±1 094	

* Per cent of standard plasma
• Reciprocal titre

The cell mediated immune responses in tonsillectomized persons were within normal limits (Table IV) This observation supports the

Table IV *Lymphocyte transformation in 9 young male adults tonsillectomized 10 years earlier*

Stimulant (µg/ml)		Tonsillectomized persons (cpm ± S D)	Control persons (cpm ± S D)
PHA	625	49 274±18 964	50 221±13 824
	125	54 196±20 797	56 821±10 855
	25	35 092± 9 109	17 189±13 797
Con A	125	17 775±12 883	36 123± 7 686
	25	32 152± 8 559	35 249±13 375
	5	16 065± 5 262	18 320± 9 009
PPD	100	2 090± 920	
	1	1 750± 1 206	
	0 01	428± 191	
Tetanus	10	1 929± 824	
	1	1 413± 807	
	0 1	816± 337	

opinion that tonsillectomy is not associated to Hodgkin's disease where the cell-mediated immunity is known to be impaired.

At present, there is no evidence that tonsillectomy has any long-term effect on immune responses. However, the indication for operation, especially in childhood, must be sufficient

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IMMUNOGLOBULIN-PRODUCING CELLS IN CLINICALLY NORMAL, HYPERPLASTIC AND INFLAMED HUMAN PALATINE TONSILS

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Abstract Patients suffering from recurrent tonsillitis (RT) hyperplastic tonsillitis (HT) or idopathic tonsillar hyperplasia (ITH) were compared in immunological studies with subjects showing clinically normal tonsils Serum concentrations of immunoglobulins particularly IgG were found to be increased in association with tonsillitis Conversely the number of IgG IgA and IgM producing immunocytes per tissue unit was reduced in the germinal centres of lymphoid follicles in the extrafollicular areas and in the reticular parts of the crypt epithelium The overall percentage distribution of these cells was normally 65 30 3 5 1 2 for the IgG IgA IgM and IgD classes respectively In RT these figures were 53 39 4 7 4 4 in HT 67 25 4 0 4 5 and in IHT 50 33 7 2 10 Thus there were only small alterations in the immunocyte class proportions except for a significant relative increase in IgD producing cells The results indicate that there is a functional defect of the tonsils in association with disease The relative accumulation of IgD cells is probably explained by an inadequate local maturational process in the B-cell system although some influence of low age cannot be excluded in the HT and ITH groups

Despite a continuing debate over the effectiveness of many if not most tonsillectomies, this operation is still the most common non-diagnostic surgical procedure in the U S and probably in many other countries (Danilevicus, 1975) There is at present no objective diagnostic method to indicate its necessity Even postoperative histopathology is of little value to this end The aim of our investigation was to study alterations in the local B-cell system associated with tonsillar disease We hoped to obtain information relevant to immunological aspects of the pathogenesis, and in addition it was of interest to evaluate immunopathological changes that might be of diagnostic value Comparative analyses of serum immunoglobulin levels were also performed Our results have been reported in detail elsewhere (Brandtzaeg, Surjan & Berdal, 1978, Surjan, Brandtzaeg & Berdal, 1978), and only a brief account will be given here

MATERIALS AND METHODS

Patient groups

The patients were classified on the basis of clinical diagnosis A control group consisted of 2 females and 6 males below 26 years of age who had clinically normal tonsils The recurrent tonsillitis (RT) group consisted of 7 females and 2 males below 26 years who for several years had continued to have two or three episodes of tonsillitis annually The hyperplastic tonsillitis (HT) group consisted of 6 females and 3 males below 20 years, and was clinically characterized as the RT group except that in addition, a marked, persistent tonsillar hyperplasia was seen The idopathic tonsillar hyperplasia (ITH) group consisted of 2 girls and 3 boys below 8 years, with persistently enlarged tonsils causing functional distress, but not showing obvious clinical signs of inflammation

Immunohistochemistry

In the control group, tissue was obtained from one of the palatine tonsils by biopsy In the other groups, specimens were obtained at tonsillectomy, which was performed in a quiescent phase of inflammatory disease The tissue

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Table I. *Serum immunoglobulin concentrations (geometric means) in four categories of subjects*

Category and age (mean range)	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgD (g/l)
Control (n = 8)[a] (14, 4–25 years)	10.79 (6.29–18.52)[b]	2.46 (0.01–8.53)	0.96 (0.41–2.24)	0.035 (0.004–0.280)
Recurrent tonsillitis (n = 9) (15, 3–25 years)	14.39 (8.79–23.54)	3.52 (1.51–8.18)	1.44 (0.62–3.31)	0.079 (0.014–0.445)
Hyperplastic tonsillitis (n = 9) (9, 4–19 years)	13.53 (8.29–22.08)	2.55 (1.01–6.45)	0.88 (0.35–2.22)	0.044 (0.004–0.529)
Tonsillar hyperplasia (n = 5) (5, 3–7 years)	10.12 (6.31–15.90)	2.02 (1.41–2.89)	0.95 (0.57–1.38)	0.051 (0.006–0.399)

[a] n = number of subjects
[b] 95% probability limits

specimens were processed for immunohistochemical localization of immunoglobulin (Ig)-producing cells by paired immunofluorescence staining (Brandtzaeg, 1974). The cells were counted in 6-μm-thick sections, and cell density was for each Ig class expressed on the basis of a defined tissue unit (section area of 0.1 mm²).

Systematic enumerations were performed throughout the section, including at-random parts of four tissue compartments: extrafollicular area, germinal centres, mantle of lymphoid follicles, and reticular parts of crypt epithelium. On this basis, cell density could be calculated for an average "tonsillar tissue unit" (representing all tissue compartments) and for a hypothetical "compartment unit".

Serum immunoglobulins

The serum concentrations of IgG, IgA, IgM and IgD were quantified by single radial immunodiffusion, and the data transformed logarithmically before comparisons were made by the Student's t-test (Brandtzaeg et al., 1978).

RESULTS

Serum immunoglobulins (Table I)

Compared with controls, RT patients had elevated levels in all Ig classes, but due to wide individual variations the difference was statistically significant only for IgG ($p<0.05$) and almost so for IgM ($0.05<p<0.1$). The same trend was observed for IgG in the HT group, whereas the other Ig classes were fairly similar to controls. The TH group showed significantly lower IgG levels and also decreased IgA, compared with RT and HT patients, but these differences might well be age-dependent.

Tonsillar immunoglobulin-producing cells

The four subject categories were compared with regard to the total number of Ig produc-

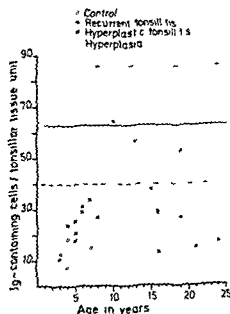


Fig. 1. Number of Ig-containing cells (all classes) per tonsillar tissue unit in relation to subject age and category. Horizontal lines indicate mean number and 95% probability limits for the control subjects.

Table II *Density of Ig containing cells in various tonsillar tissue compartments related to clinical diagnosis*

Patient category	Mean cell number (all classes) ± S D per compartment unit			
	Germinal centre	Mantle of follicle	Extrafollicular area	Reticular epithelium
Control	33 2±12 9	10 9±5 6	90 2±35 7	228 1±59 1
Recurrent tonsillitis	16 7±16 9*	9 4±7 8	30 1±17 2*	181 1±98 7
Hyperplastic tonsillitis	24 8±15 3	8 2±7 6	34 3±18 6*	116 5±65 7*
Tonsillar hyperplasia	11 1± 5 9*	6 6±4 6	18 0± 9 3*	91 9±53 5*

* Significantly different from the control

ing cells per tonsillar tissue unit IgE immunocytes were excluded from these calculations, since only an occasional cell was found The immunocyte density of all except two diseased tonsils was below the 95% probability limit of the control group (Fig 1) The various Ig classes were not equally represented in this decrease Thus, the number of IgD-containing cells either remained stable or showed a slight increase, especially in ITH, whereas the reduction was statistically significant for the three other immunocyte classes—except when the standard deviation was extremely large (Fig 2)

In all disease categories the decrease in Ig-producing cells was most marked for the extrafollicular area, but it was significant also for the germinal centres in the RT and ITF groups, and for the reticular epithelium in the HT and ITH groups (Table II) The lowest overall immunocyte density was seen in the ITH group

Only small changes in immunocyte class ratios were observed for the average tonsillar tissue unit, excepting a significantly increased proportion of IgD-containing cells (Table III) This increase was especially uniform in the extrafollicular area and reticular epithelium, al though in some cases the ratio of IgD immunocytes was strikingly elevated also in the two other tissue compartments

DISCUSSION

It is difficult to compare our results with information obtained in previous studies, since these have been performed without systematic reference to clinically normal tonsils In his pioneer report on tonsillar plasma cells, Davis (1912) described greatly increased numbers in pathological specimens where the cells occurred in clusters and sheets, especially beneath the epithelium It should be noted that the connective tissue adjacent to the faucial surface epithelium was excluded from our quantitation of Ig-containing cells We focused

Table III *Class distribution (%) of tonsillar Ig producing cells related to clinical diagnosis*

Ig class of immunocyte	Mean percentage ± S D			
	Control	Recurrent tonsillitis	Hyperplastic tonsillitis	Hyperplasia
IgG	65 2±11 8	52 5±10 5	66 5±7 8	49 5±11 6*
IgA	30 1±11 7	38 6±11 3	25 0+8 0	33 3± 9 9
IgM	3 5± 2 0	4 7± 3 5	4 0±2 0	7 2± 3 3
IgD	1 1± 1 0	4 4± 5 1*	4 5±3 4*	10 1± 6 8*

* Significantly different from the control (Wilcoxon test)

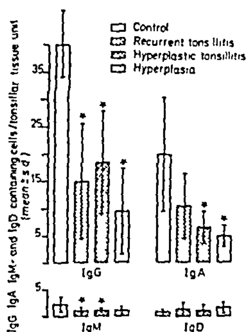


Fig. 2. Number of Ig-containing cells of various classes per tonsillar tissue unit in the four subject categories. Asterisk indicates statistically significant difference from the control group.

on the compartments of lymphoid elements, and a defect in their function was indicated by the decreased density of Ig-producing cells.

The previously most detailed immunohistochemical study of human tonsils was carried out by Ishikawa, Wicher & Arbesman (1972). They found IgE-containing cells in 28% of the specimens, whereas such immunocytes were virtually absent from our material. Östergaard (1975) reported IgE cells to be present in 40% of diseased tonsils, but technical difficulties must be seriously considered in the localization of such cells (Brandtzaeg & Baklien, 1976). In agreement with our findings, Ishikawa et al. (1972) observed that the IgG-producing cells always predominated.

In patients with RT, the serum levels of all Ig classes were elevated, and significantly so for IgG. This is in agreement with the report of Veltri et al. (1972), they concluded from microbiological studies that RT is a viral bacterial disease (Sprinkle & Veltri, 1976). There is likewise evidence to suggest that chronic infection can cause elevated IgD levels (Buckley & Fiscus, 1975). Conversely, some investigators have proposed that a slight deficiency of serum IgA may underlie RT and predispose to infection, especially with *Haemophilus influenzae* (Donovan & Soothill, 1973, Östergaard, 1976). Östergaard (1975) moreover claimed that this deficiency might be manifested in the tonsils by a complete lack of IgA-producing cells in 30% of the patients. Also Nezelof et al. (1974) reported absence of tonsillar IgA and IgG or IgM cells in 17% of young children with HT, despite the concurrent presence of normal immunoglobulin levels in serum. If these findings were obtained with representative tissue specimens, they are clearly different from our results which showed a decreased density but never an absence of Ig producing cells in diseased tonsils.

The overall decrease of the immunocyte density in inflamed tonsils probably reflects a lowered functional capacity in the various tonsillar lymphoid compartments. Thus fibrosis was frequently observed in the extrafollicular area. It would moreover be of interest to know whether the antigen absorptive activity of the reticular epithelium is decreased, if so, retarded clonal expansion and inadequate differentiation of local B cells could be expected. Since such adverse alterations may be secondary to inflammation, our study does not allow a final conclusion with regard to a possible local defective immunological function underlying tonsillar disease. To what extent a possible lack of tonsillar "helper" T cells (Baumohl et al., 1977) might have contributed to the observed reduction of activated B cells is unknown. IgD seems to play a role during the initial triggering of unprimed B cells (Cooper et al., 1976), and the demonstrated relative accumulation of IgD-producing cells in diseased tonsils may be compatible with lack of proper stimulatory signals for clonal maturation.

It may be argued that our control material of clinically normal tonsils from the younger age group was not appropriate. With regard to age it should be satisfactory for comparisons with the RT group, but the subjects were not

matched for sex This reflects the difficulties in obtaining tonsillar control material, a problem that has been pointed out in most preceding studies These difficulties are even more pronounced when it comes to matched controls for patients with hyperplastic tonsils at ages below 8 years Our findings in the HT and ITH group must therefore be interpreted with caution The results obtained were principally similar to those in the RT group Nevertheless, tonsillar hyperplasia with low immunocyte density may well be a normal situation in the very young The ITH group showed the highest proportion of IgD producing cells, and this might indicate immaturity of the local B cell system

Our study clearly indicated decreased immunological function of the lymphoid tissue in diseased tonsils This observation is too preliminary, however, to be of any help in the debate about the justification of tonsillectomy Its diagnostic value might seem promising but the quantitative immunohistochemical method used in this study would be too cumbersome for a practical diagnostic approach Further studies along similar lines are obviously needed in combination with careful longitudinal clinical evaluation of the patients

ACKNOWLEDGEMENTS

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T CELLS AS MARKED WITH ACID α-NAPHTHYL ACETATE ESTERASE STAINING IN SECRETORY OTITIS MEDIA

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Abstract Non specific acid α naphthyl acetate esterase (ANAE) activity of the lymphocytes was investigated in smears made of middle ear secretions and peripheral blood from 61 children with secretory otitis media On the average, 20 1% (right ear) and 21 1% (left ear) of the lymphocytes in the ear samples showed distinctive ANAE positive spots in their cytoplasms, by the method used The mean percentage of ANAE positive cells in the blood was 47 8 and it was significantly ($P<0\ 001$) higher than the respective percentages of the ear samples In normal children, 54 9% of the lymphocytes on the average were ANAE positive This difference in blood between patients and control group was significant ($P<0\ 001$) Consequently, the present study suggests that the relationships of T and non T lymphocytes as marked with ANAE, are disturbed in children suffering from secretory otitis media The importance of this finding and the validity of the method are discussed

Secretory otitis media (SOM) is the most prevalent otological problem in childhood Its etiopathogenesis, however, despite innumerable studies, is far from having been resolved Although mechanical reasons probably explain a certain proportion of the cases, the increase in the frequency of SOM during recent decades favours the theories suggesting allergic (Derlacki, 1952) or infectious (Senturia et al , 1958) origins of the disease However, studies concerning allergic phenomena in the middle ear are contradictory and attemps to show anti infectious immunological defence systems to be defective, have, as yet, failed

Because earlier studies have mainly concentrated on antibody-mediated immunological mechanisms, and because it has been proposed (Bernstein, 1976) that delayed hypersensitivity may play a part in SOM, we decided to examine the cell mediated defence systems of SOM by studying the occurrence and role of T cells as marked with non specific acid α-naphthyl acetate esterase (ANAE) staining (Mueller et al , 1975) in the secretions and peripheral blood in children with SOM

MATERIAL AND METHODS

Middle ear specimens were taken in connection with tympanostomy from 86 ears of 61 children with secretory otitis media The mean age of the children was 4 4 years (range 0 5–11 4 years), there were 18 girls and 43 boys The symptoms of SOM had lasted, on the average, for 3 (range 1–7) months Twenty of the ears had been aerated earlier but the tube had extruded None of the patients had signs of acute infection or received antibiotic therapy

Simultaneously with the ear samples peripheral blood was drawn Control blood samples were taken from 76 children, 31 girls and 45 boys, with the mean age of 5 2 (range 0 1–15 4) years None of them had any infections or a systemic disease

The effusions aspirated were always gluelike, and they were immediately washed three times with RPMI-1640 (Gibco Bio-Cult Ltd , Scotland) and cytocentrifuged onto slides The blood samples were directly smeared on object glasses Both were then left to dry at room temperature, and the procedure was continued using the method of Mueller et al (1975) with some modifications After the fixation with

This study was supported by grants from the Emil Aaltonen Foundation Finland and the Paolo Foundation Finland



Fig 1 Middle ear specimen showing one lymphocyte with a distinct ANAE positive spot (*arrow*) and two negative lymphocytes. A macrophage (*M*) stains strongly and diffusely. ×1000

Baker's solution for 10 min at 4°C specimens were washed in distilled water at room temperature for 20 min and thereafter incubated with the ANAE marker at 37°C for 3 hours at pH 5.8 (Kulenkampff et al., 1977). After washing in distilled water for 10 min the slides were counterstained with 1% aqueous toluidine blue for 60 min (Ranki et al., 1976). The ANAE positive lymphocytes were easily recognizable by a single or a few distinct reddish brown spots in their cytoplasms (Fig 1), while the cytoplasms of monocytes and macrophages became diffusely reddish-brown (Fig 1). The percentage of ANAE-positive cells of the total number of lymphocytes was calculated in each specimen.

Additional smears from 84 ear and 60 blood samples were stained with May-Grünwald-Giemsa (MGG) to count the percentage distribution of different cellular types in these specimens. The bacteriological samples from 66 ears were taken with a sterile glass suction tip and cultured on chocolated blood agar plates.

The statistical analyses were done as follows: Significance with the Student's *T*-test;

Table I *Per cent cell distribution in effusion and blood*

	No of samples	Neutrophils		Lymphocytes		Macrophages		Monocytes		Eosinophils	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Right ear	44	52.6	19.6	23.7	11.7	14.3	11.6	9.1	5.3	0.3	0.8
Left ear	40	51.4	20.1	25.4	12.8	14.0	12.8	8.6	4.8	0.1	0.4
Blood	60	49.0	10.9	44.3	10.8	–	–	4.8	2.4	1.8	1.4

Table II *Percentages of ANAE positive cells*

	No of samples	Mean	S D
Right ear	45	20 1	12 3
Left ear	41	21 1	13 5
Blood	61	47 8	12 2
Blood of controls	76	54 9	6 8

the effect of age and sex with covariance analysis, and correlations by defining Pearson's correlation coefficient

RESULTS

Table I shows the types of cells in the effusions and blood samples In the effusions neutrophils and lymphocytes dominated Macrophages and monocytes also occurred abundantly. Eosinophils and basophils (mast cells) were very few In some ear samples epithelial cells could also be found Cellular remnants were few in 30 samples, moderate in 18 and abundant in 36

The mean percentage of ANAE-positive cells of total lymphocytes in the bloods of the patients was 47 8 (Table II) In the control group the corresponding percentage was 54.9 When standardized with regard to age and sex, the difference between the two groups was significant ($P<0\ 001$) Additionally, the mean percentage of ANAE-positive cells in the effusions was 20 5 and it was also significantly lower ($P<0\ 001$) than the respective percentage in the bloods of the same patient group A strong positive correlation was found between the numbers of ANAE-positive cells in the right and left ear

Basophils		Epithelial cells	
Mean	*S D*	*Mean*	*S D*
0 1	0 3	0 1	0 1
0 1	0 2	0 1	0 4
0 2	0 2	-	-

Table III. *Bacteria cultured*

	No of cultures
Staphylococcus aureus	5
Staphylococcus epidermidis	3
Diplococcus pneumoniae	2
Branhamella catarrhalis	1
Haemophilus influenzae	1
Klebsiella sp	1
Negative	53
Total	66

Bacteria could be cultured in 24.7% of the samples (Table III) Most of them were staphylococci

DISCUSSION

It has been suggested that T cell dependent mechanisms play an important role in the protection of the middle ear from infection (Bernstein, 1976) However, the direct data about T cells in SOM is scanty The reason for this is probably the difficulty of identifying T lymphocytes in rather viscous effusion of SOM, which excludes the use of commonly accepted functional methods Non-specific acid α-naphthyl acetate esterase staining marks rather specifically human T lymphocytes directly from cell smears (Ranki et al , 1976) and is applicable for glue secretions as well as for blood

The present study showing the proportion of T cells of total lymphocytes in the effusions of SOM to be significantly reduced when compared with their distribution in blood is consistent with out preliminary results (Sipila et al , 1978), but contrasts with those of Palva et al (1978) However, it must be remembered that the method used is rather delicate and even small changes in some technical details may alter results remarkably (Kulenkampff et al , 1977) Consequently, our findings suggesting the local T cell defect must be received with caution until the results have been confirmed in other laboratories and by the same and by other methods However, parallel with the above finding was that the percentage of T

cells was significantly reduced in the bloods of the patients as compared with age and sex-matched controls Analysis of T cells in both groups was done by exactly the same technique and by the same persons and therefore this difference must be considered of importance

Allergy has been claimed (Derlacki, 1952) to be the cause of SOM On the other hand, it has been shown that the number of T cells is decreased (Strannegård et al, 1976) in atopics This decrease is proposed to be due to a defect in suppressor T cells (Strannegård et al, 1976), which results in hyperproduction of IgE and atopic disease Although reports concerning the IgE levels in the sera and effusion of SOM are contradictory, it has been shown that the middle ear mucosa can act as an allergenic shock organ (Miglets, 1973) and can synthesize IgE (Hussl & Lim, 1974) Consequently, our finding of a decreased proportion of T cells in the blood of patients with SOM is in line with the idea that allergy may predispose to SOM Further, the still more decreased percentage of T cells in the effusion of SOM may mean that in these children T and B cell interactions are locally still more disturbed making the middle ear especially prone to injury

The cellular picture of the effusions with the dominance of neutrophils and lymphocytes favours the infectious origin of the disease The frequent occurrence of monocytes and phagocytes confirms this idea and suggests an effort to overcome the inflammatory state of the middle ear by their ability to eliminate foreign material and by their co-operative role in immunological defence systems The relatively rare occurrence of pathogenic bacteria on the other hand hints that the inflammatory state possibly primarily caused by pathogenic bacteria is partly overcome but still persists in a latent form or it is caused by low-virulence bacteria Also these findings, together with the shortage of T cells, do not exclude the possibility that viruses may be involved in SOM

Summarizing the present study suggests that in children with SOM the distribution of T and non-T cells in the effusion and blood is disturbed by the relative shortage of T cells The T cell mediated immune defence system seems to be depressed and this may lead to reduced resistance against inflammatory processes the end of which is SOM

ZUSAMMENFASSUNG

Die unspezifische saure α Naphthyl Acetat Esterase (ANAE)-Aktivitat der Lymphozyten wurde in Ausstrichpraparaten aus Mittelohrsekretionen und dem peripheren Blut von 61 Kindern mit sekretorischer Mittelohrentzundung studiert Mit der angewandten Methode zeigten im Durchschnitt 20 1% (rechtes Ohr) und 21 1% (linkes Ohr) von den Lymphozyten der Ohrproben eindeutig ANAE positive Punkte in ihren Zytoplasmen Die durchschnittliche Prozentzahl von ANAE positiven Zellen im Blut war 47 8 und sie war signifikant höher ($P<0\ 001$) als die entsprechenden Prozentzahlen der Ohrproben Bei normalen Kindern waren im Durchschnitt 54 9% der Blutlymphozyten ANAE-positiv Diese Differenz zwischen den Blutproben von den Patienten und von der Kontrollgruppe war signifikant ($P<0\ 001$) Aus dem Ergebnis der vorliegenden Untersuchung kann man schließen daß das Verhaltnis von T und nicht T Lymphozyten bei Kindern mit sekretorischer Mittelohrentzundung gestort ist wenn die Lymphozyten mit der ANAE Färbung markiert sind Die Bedeutung der Ergebnisse und die Zuverlassigkeit dieser Methode werden diskutiert

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Fig 1 Immunohistochemical localization of IgG (*a*) and IgA (*b*) in Warthin's tumour The specimen had been extracted in saline before fixation to remove diffusible immunoglobulins Comparable fields in adjacent sections show a group of Ig producing cells (*open arrow*) beneath the tumour epithelium (*ep*) Note predominance of IgG immunocytes There is a selective retention of IgA along the luminal border of the epithelium and apically in some of the luminal epithelial cells (*arrows*) Manification ×150

were found to contain a few Ig producing immunocytes, mainly of the IgM class Scattered in the extrafollicular lymphoid tissue were Ig-producing cells of all classes except IgE, mostly IgG cells (Fig 1 and Table I) Paired staining for κ and λ light chains showed type restriction to individual cells

The epithelium

Many of the luminal cells showed apical staining, apparently representing cytoplasmic SC and IgA (Fig 2) Faint intercellular staining for these components was seen in some parts of the epithelium, and the amorphous material present in the cystic spaces was also brightly stained (Fig 2) CEA was not seen in ducts of normal parotid tissue, but was found in small patches of the tumour epithelium, mainly in the scattered papilliferous projections (Fig 3)

Table I *Number and class distribution of immunoglobulin-producing cells in Warthin's tumour*

Immunocyte class	Ig producing cells/mm² tissue section area: Median (range)	Percentage
IgG	20 4 (15 2–27 2)	68
IgA	4 8 (2 8–12 4)	16
IgM	4 8 (0 4–9 6)	16
IgD	0 (0 0–1 2)	0

DISCUSSION

Adenolymphomas are benign tumours of the parotid gland, 82% occurring between the age of 40 and 70 years (Chaudhry & Gorlin, 1958) Among the benign parotid tumours observed at Rikshospitalet (Berdal et al, 1970), 4 8% were adenolymphomas which were found next

Table II *Comparison of the class distribution of immunoglobulin-producing cells in Warthin's tumour, normal palatine tonsils and normal parotid tissue*

	Percentage class distribution of Ig producing cells			
	IgG	IgA	IgM	IgD
Warthin s tumour	62 1	20 3	16 5	1 1
Palatine tonsil[a]	65 2	30 1	3 5	1 2
Parotid gland[b]	3 6	90 7	3 1	2 6

[a] From Brandtzaeg et al (1978)
[b] From Korsrud & Brandtzaeg (1978)

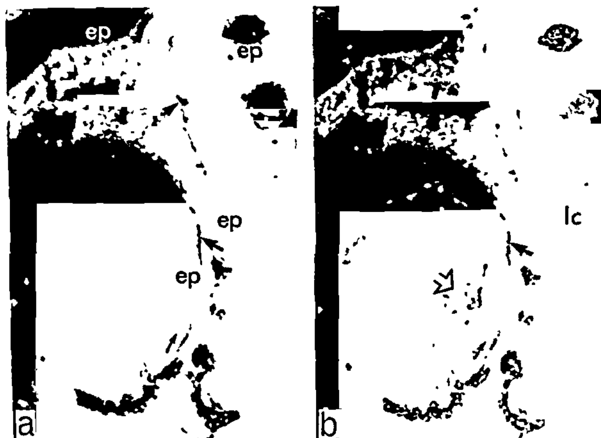


Fig 2 Paired immunohistochemical staining with rhodamine conjugate specific for SC (*a*) and fluorescein conjugate specific for IgA (*b*) in a section of directly alcohol fixed Warthin tumour tissue The two pictures from this field show that SC and IgA are localized together intercellularly (*small arrows*) and apically (*large arrows*) in the tumour epithelium (*ep*) and in the amorphous material present in the cystic spaces The dark stromal areas represent the lymphoid component (*lc*) of the tumour and a group of IgA producing immunocytes is indicated by an open arrow Magnification ×150

in frequency to pleomorphic (54 0%) and other adenomas (5 2%)

The works of Thompson & Bryant (1950) and Azzopardi & Hou (1964) record clear morphological evidence of a developmental origin of this tumour Due to the poor separation between the parotid and lymphoid tissue in this area during fetal development, the tumour *may originate in the parotid gland itself* (Berdal et al , 1970) or in lymph nodes in the preparotid area (Azzopardi & Hou 1964)

Allegra (1970), on the contrary, believes that the tumour may reflect a hypersensitivity condition primary oxyphilic metaplasia of the ductal epithelium is followed by proliferation of the ductal elements tion and subsequent cyst formation, stimulating an intense lymphoid cellular infiltration There is, however, no direct evidence for his hypothesis and the morphology of the lymphoid tissue is quite compatible with normal lymphoreticular tissue (Thompson & Bryant, 1950, Azzopardi & Hou, 1964) Our immunohistochemical investigation supports the latter *view and demonstrates a marked similarity between* the lymphoid stroma of Warthin's tumour and, for example, tonsillar tissue with regard to the class distribution of Ig producing cells, which contrasts sharply with that found in normal parotid tissue (Table II). However, the number of Ig producing cells is lo

tumour than in the tonsillar tis

All



Fig 3 Immunohistochemical localization of carcino-embryonic antigen in a papilliferous patch (*arrow*) of the tumour epithelium (*ep*) in a directly alcohol fixed specimen of Warthin s tumour There is also staining of cellular debris present in the cystic space Magnification ×390

ing a more pronounced activation of the B cell system in the tonsil The presence of lymphoid follicles with large mantle zones containing IgD- and IgM-bearing lymphocytes is in agreement with the recent study of Cossman et al (1977) which indicates a preponderance of B lymphocytes in the tumour stroma

Localization of SC and IgA in the luminal epithelial cells of Warthin's tumour is comparable to that found in normal striated duct cells, suggesting a ductal origin of the tumour epithelium This is in agreement with previous morphological evidence of a developmental origin of Warthin s tumour (Thompson & Bryant, 1950, Azzopardi & Hou, 1964) The presence of CEA in the pailliferous epithelium may indicate some degree of de differentiation in these projections

In conclusion, our study indicates that Warthin's tumour is composed of a normal but not highly activated lymphoreticular tissue and a neoplastic duct epithelium of fairly high differentiation as shown by its IgA transporting capacity

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SUPPLEMENT 361

Pathogenesis and Surgical Treatment of the Middle Ear Cholesteatoma

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SUPPLEMENT 361

Pathogenesis and Surgical Treatment of the Middle Ear Cholesteatoma

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LUZIUS RÜEDI

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To my strongest opponent
but most admired and
dearest American friend
George E. Shambaugh, Jr.

Abstract

After confirmation of Manasse s and Lange s findings by O Steurer W Albrecht and M Schwarz and our statement of errors in Wittmaack s doctrine Bezold s retraction theory has been abandoned by most of the German reading otologists

The effects exerted by infections on the normal development of the middle ear spaces have been studied In review of these results and combined with new observations by David J Lim Herbert G Brick and W Saunders an identical pathogenesis of all acquired cholesteatomas seems probable

1 Every cholesteatoma is composed of a matrix originating from the basal cells of the epithelium of the tympanic membrane or of the drum adhering meatal skin and out of a mesenchymal substrate the perimatrix (D J Lim and co-workers) which furthers and leads the growth of the matrix

2 This growth takes place after the destruction or opening up of the basal membrane

3 The inflammation which accompanies the infection of the middle ear—and occasionally a chronic eczema of the meatal canal—stimulates the potent basal cells to grow with solid cones (matrix) through the defective basal membrane into a mesenchymal substrate (perimatrix)

4 The perimatrix consists of inflamed subepithelial connective tissue or of new granulation tissue In the attic cholesteatoma the perimatrix is formed by submucous layers of loose connective tissue due to incomplete pneumatization

5 The active growth of the matrix stops on reaching the bone Granulation tissue covers the denuded bone Bruce J Gantz and his collaborators and Maxwell Abramson and his colleagues found bone resorbing enzymes in this granulation tissue

6 There then starts a second more passive phase of cholesteatomous growth The desquamation of epithelial lamellae continues and the cholesteatomatous sac enlarges While M Abramson does not accept that a pressure effect of the cholesteatomatous material is involved in the bone resorption I am still convinced that continuous pressure changes within the cholesteatoma masses stimulate the bone resorbing activity of the granulation tissue covering the bone

7 During these two phases of cholesteatoma growth some tubes break into the middle ear cavity and through Shrapnell s membrane forming an attic performation It is behind such perforations that large cholesteatomas will mostly be found

The treatment of every cholesteatoma must eliminate the entire matrix and also the total perimatrix

As soon as the cholesteatomatous tubes have reached the posterior wall of the tympanon only an open cavity technique can render the necessary cleaning of the tympanic cleft and the communicating cell system possible The high recurrence rate after a canal up posterior–superior tympanotomy is attributable to incomplete elimination of matrix and especially of perimatrix rather than to a malfunctioning of the Eustachian tube

The incidence of attic cholesteatomas might be reduced by a preventive operation in suspect cases

Introduction

George E Shambaugh, Jr, in the first edition of the leading American textbook on the *Surgery of the Ear* (1959), subdivides middle ear cholesteatomas conventionally into secondary acquired cholesteatomas, primary acquired cholesteatomas, and congenital cholesteatomas

This last disease has nothing to do with chronic suppurative otitis media Due to an epithelial remnant of embryonal origin, it can also occur in the temporal bone, though it must always be uninfected and separate from the middle ear cleft Fulfilling these conditions, the congenital cholesteatoma is so rare as to be neglected in this survey

J Habermann, early in 1888, was the first to observe the invasion of the middle ear by squamous epithelium of the external canal, bordering superiorly upon a large perforation of the tympanic membrane after a necrotizing otitis media in infancy By means of a continuous desquamation of epithelial lamellae, ingrown skin produces within the mid ear cavity a cholesteatoma Shambaugh follows Habermann's concept, although the decisive discovery, still valid for all types of acquired cholesteatomas, was made by P Manasse (1917) Manasse showed that the ingrowing squamous epithelium of the meatal skin has a strong tendency to proliferate Not content to advance on the surface as a healing process, it spreads mainly by sending forth strands and cones of basal cells into the submucucous connective tissue of the still intact cubical epithelium of the middle ear

Besides the sudden onset of a secondary acquired cholesteatoma after necrotic otitis media, another form of cholesteatoma was discovered, slow-spreading and symptomless, characterized by a perforation of Shrapnell's membrane, quoting again Dr Shambaugh

"The etiology of primary acquired cholesteatoma has been the subject of much debate Bezold explained the development of the primary acquired variety as the result of tubal occlusion, leading to a retraction of Shrapnell's membrane into the attic, where its outer cornified layers proceeded to collect as a plug of epidermal debris Wittmaack enlarged upon this theory by showing that a persistence of hyperplastic embryonic type of mucoperiosteum in the epitympanon could lead to adhesions, walling off areas especially between the head of the malleus and Shrapnell s membrane (Prussak's space) Since air from the Eustachian tube could not reach these areas, the resulting negative pressure would have the same effect as prolonged tubal occlusion "

"While the explanations of Bezold and Wittmaack agree with the clinical observations of the author (Shambaugh) and have received widespread acceptance, other theories are still held " Tumarkin believes in metaplasia of the middle ear mucosa, McKenzie, Diamant and Teed believe that a congenital epidermal remnant causes most cases of attic cholesteatomas that occur with a perforation, confined to the pars flaccida Nager, Lange, Hellmann and others, and more recently Saxen & Ojala and Ruedi, have modified Wittmaack's theory by stating that cone-like extensions from the basal layer of epidermis of Shrapnell s membrane can become invasive as the result of infection, producing firstly a cholesteatoma in the attic and then later a perforation of Shrapnell's membrane outward to the external auditory canal

The simplest theory, that of Bezold and Wittmaack, namely that the entire process begins as a simple pouch like invagination of Shrapnell s membrane in response to negative pressure in the attic, seems to be adequate

to explain the clinical manifestations of attic cholesteatoma that arise without necrotic otitis and without a pars tensa perforation

George Shambaugh then describes the successive stages in the development of a primary acquired cholesteatoma "The first stage starts with a dimple like identation of the pars flaccida which (1) might be the result of an infantile otitis media, before the fetal subepithelial connective tissue of the middle ear of the newborn child has had time to recede (Wittmaack's suggestion of infantile sterile otitis media neonatorum), and (2) a permanent pit like retraction of the pars flaccida may develop from a longstanding secretory otitis media "

The *second stage* occurs "if the pit like retraction of the pars flaccida becomes filled with a plug of desquamated epithelium the pressure from added layers of desquamating epidermis causes the pocket to enlarge, slowly at first, and more rapidly when the debris becomes contaminated by bacteria from the external meatus'

"When the pocket reaches the walls of the attic, it proceeds to enlarge by bone erosion in exactly the same manner as the secondary acquired cholesteatoma In some cases, the expanding sac like cholesteatoma pocket remains above the ridge of the bony tympanic facial canal, in others it may project down to the tympanic cavity as a small epidermal sac, filled with desquamated debris and entirely separate from the mucous membrane-lined air space of the tympanic cavity Inflation of the Eustachian tube in such a case will cause the pars tensa to bulge without the escape of air from the epitympanic opening Should the skin lined sac rupture, spilling its infected content into the tympanic cavity, an acute otitis media will occur The physician should not conclude that the cholesteatoma is the result of the acute otitis media "

The opening from the external auditory meatus into the cholesteatoma cavity appears to the examiner as a hole or perforation of the pars flaccida As we have seen from the manner it develops, it is in reality not a perforation at all, but an *invagination of the pars flaccida* which expands as the cholesteatoma cavity enlarges "

I have copied Shambaugh's description of the development of a primary acquired cholesteatoma because I have for 3 years, made weekly inspections of the tympanic membrane and especially the pars flaccida, on the wards of our Paediatrics Department, in children between 1 and 5 years of age—irrespective of their disease I have seen a certain number of retracted Shrapnell membranes with some scarring signs and a few with a bulging Shrapnell s membrane, due to an acute epitympanic otitis media But I have never seen what I was looking for the just described development of primary acquired cholesteatoma out of a retracted Shrapnell membrane Whenever I have found a suspect granulation and some secretion in the region of the pars flaccida or a small, sometimes dry perforation, it has been possible, using a small hook and a magnifying loop or the otomicroscope, to obtain cholesteatomatous material Surgery in such seemingly early cases has often revealed a surprisingly large primary acquired cholesteatoma Dr Sade (Jerusalem) has reported similar experiences

I am still puzzled by three questions

(1) Has the development of a primary acquired cholesteatoma been followed up in in one and the same patient?

(2) How is the passively insucked, stretched, and in some cases ruptured epidermis of Shrapnell s membrane converted into an actively growing cholesteatomatous matrix?

(3) One of the milder ear diseases is so called catarrh of the Eustachian tube which, as a result of oxygen resorption (?) causes a retraction of the tympanic membrane and more often a visible accumulation of a serous liquid, the so called "hydrops ex vacuo" Today these cases heal mostly after paracentesis and insertion of a v

lating tube But has the development of a Shrapnell's pocket, transforming into an attic cholesteatoma ever been observed in these oft-recurring cases?

In the second edition of *Surgery of the Ear* (1967), instead of 'primary acquired cholesteatoma', Dr Shambaugh consequently uses the name 'attic retraction cholesteatoma', a rather frequently encountered variety of chronic suppurative otitis media in which perforation—often very small—is confined to the region of the pars flaccida of the tympanic membrane Between 1930 and 1939, even in Germany, the origin of the so-called primary acquired cholesteatoma and the appearance of Shrapnell s perforation was discussed The importance of Manasse's discovery (1917) was not recognized, whereas K Wittmaack's (1918) simultaneously published concept of the 'normal and pathological pneumatization of the petrous bone' was immediately accepted and introduced in most textbooks Due to Wittmaack's very aggressive personality, his doctrine inhibited during his lifetime not only an appreciation of Manasse's findings, but also further contributions of younger otologists, concerning the pathogenesis of primary acquired cholesteatoma

As early as 1925, W Lange had furnished fundamental observations the histological proof of deeply ingrowing prickle cells out of the epidermis of Shrapnell s membrane, which was neither indrawn nor perforated He considered that this epithelial proliferation was caused by a mild inflammatory stimulus such as a prolonged attack of acute otitis media In Lange's opinion, these proliferating columns of basal cells grow into the connective tissue of Prussak s space, thus forming the basis of a Shrapnell cholesteatoma

A Albrecht, O Steurer and M Schwarz have pointed out the importance of connective tissue rests in the epitympanic space as a substrate for the start of a primary acquired cholesteatoma Therefore Bezold s hypothesis of a chronic tubal catarrh, leading to retraction and final rupture of Shrapnell's membrane, finds little support today in the German literature Encouraged by my former chief, Prof F R Nager, I made a statistical analysis of 763 cholesteatomas, seen at the Zurich University E N T Clinic between 1918 and 1934 631 were secondary acquired cholesteatomas with intracranial complications in 1 7%, whereas in the smaller group of 132 Shrapnell cholesteatomas the incidence of intracranial complications was 6% (four times higher) Secondary acquired cholesteatomas started as a necrotizing otitis of infancy in 35% arising in the course of scarlet fever, measles, diphtheria, tuberculosis or influenza, and producing large upper marginal perforations or total defects of the tympanic membrane In the remaining 65% no history could be obtained, but in the majority of these cases, the middle ear infection dated from early childhood Therefore it may be assumed that these cholesteatomas also began as a necrotizing otitis media

This assumption does not hold good for a small group of 11 cases with small perforations in the postero superior region of the pars tensa, neighbouring the bony border In 59% of this small group, severe complications occurred (1 facial palsy, 3 extradural abscesses, 2 meningitis with two deadly brain bascesses) The origin of these cholesteatomas with small postero-superior perforations was unknown in 1934

Meanwhile I have seen 4 cases (a 1-year-old boy, a 15-year old boy, an 18 year old girl and a 35-year old man) with small postero superior perforations In 2 of these cases (the 1-year-old boy and the 15-year-old boy), during a subacute mastoiditis of the right ear, the pus perforated directly into the external auditory meatus, posteriorly-superiorly in front of the tympanic membrane In the case of the 1 year old boy, the ingrowing squamous epithelium of the external meatus could be demonstrated histologically after the mastoidectomy The 18-year-old girl very probably had an infantile scarlatine otitis media At the

age of 17, secretions out of the right ear occurred One year later, a small postero-superior perforation of the tympanic membrane was discovered With a conservative operation, an ingrowing cholesteatoma could be eliminated The 35 year old man was brought to the clinic unconscious, with symptoms of otogenic meningitis He had a small postero superior perforation of the tympanic membrane, filled with granulation tissue Shortly after admission the patient died Histological examination of the petrous bone showed a cholesteatoma growing with small tubes through inflamed, submucous connective layers within the epitympanic space to the dura mater, producing an extradural abscess, meningitis and a brain abscess

In the Zurich series of 132 so called Shrapnell cholesteatomas, only 20% reported a history of otitis media in early infancy In none of these cases did an ear specialist observe the appearance of a Shrapnell s perforation in the course of acute otitis media Nevertheless, a few cases of Shrapnell membrane [illegible]

case of acute otitis media complicated by a brain abscess, where a puncture of Shrapnell s membrane was observed Four weeks after the death of the patient, histological examination showed the onset of a secondary acquired cholesteatoma Epithelial cones from the Shrapnell epidermis, ingrowing through no longer visible puncture, had reached the antrum I have seen and published a similar case as probably the rarest form of a secondary acquired cholesteatoma

Due to preventive inoculation therapy and the regular administration of sulphonamides and antibiotics for severe infantile infections, necrotizing otitis media and consequently secondary acquired cholesteatoma have nearly disappeared in medically well developed countries The still important number of secondary acquired cholesteatomas, existing since childhood, seen and operated upon in Switzerland within the last 15 years, were found mainly in foreign labourers coming from southern countries Probably within some of the underdeveloped regions the number of ears affected by necrotizing otitis media and consequently the occurrence of secondary acquired cholesteatomas will not decrease for many years

Between 1934 and 1936, in order to check up on the teaching of Wittmaack, I have examined the normal development of the middle ear spaces between the fifth month of fetal life and 10 years of age in a large series of temporal bones This study does not support Wittmaack's theory of pneumatization (Fig 1) The normal development of the air spaces is more complicated and has many more variations than assumed by Wittmaack In the embryonic stages, the formation of the middle ear spaces proceeds in two different processes In the first process (Fig 1, upper curve) the main bone spaces of the middle ear are differentiated within a fibrous cartilaginous bony mesenchyme and at about the seventh fetal month a widely varying anlage of cells starts from the antrum into the growing bone These cells are completely filled with connective tissue In a second process (lower curve) these middle ear spaces, preformed in the bone, are pneumatized by an ingrowing strand of mucous membrane emanating from the Eustachian tube This process of pneumatization also shows individual variations in speed (indicated by arrows) Accordingly, several possible combinations of individually varying preformation of bone spaces and their pneumatization (which also proceeds at an individual pace) will be found at the time of birth Four of the many possible combinations are shown in Fig 2

These sections run vertically on identical planes through the small antrum of four newborns without ear pathology In case 1, only a small antrum has developed which is filled by loose connective tissue In case 2, the equally small antrum is pneumatized In case 3, there is a large antrum with cells originating

from it In all these spaces, remaining connective tissue is found (Fig 3) Case 4 demonstrates a large antrum Its numerous cells are almost completely pneumatized

The further process of middle ear cavity formation has been histologically examinated in 31 petrous bones of children between birth and 10 years of age, without a history or any signs of ear disease

In these normal cases, within the first year, the second process of pneumatization (Fig 1, lower curve) arises with the normally varying progress of bone cavity formation (upper curve), due to the also varying disappearance of the initially separating connective tissue

From now on a thin mucous membrane lines all the spaces and cells of the middle ear But as seen at birth, further associated pneumatization within the growing mastoid and petrous bone, following typical routes, first described by George Portmann, continues in healthy ears (contrary to the description of Wittmaack), normally varying between very extensive and smaller cell formations

When we examined the effects exerted by different kinds of otitis media on the development of the middle ear spaces, we reached conclusions contrary to Wittmaack Otitis 'a neonatorum, due to aspiration of meco- does not affect the two processes of middle ear cavity formation Infectious acute otitis media occurs mostly in infants with a prolonged association of the two cavity building processes Due to the resulting persistence of submucous connective tissue, these infants are susceptible to infectious inflammation of this congenital hyperplastic tissue A narrowed or closed Eustachian tube inhibits aeration and therefore favours the duration and recurrence of disease, can be seen in Fig 4

Acute otitis media produces only a cessation of pneumatization, whereas the preformation of cells within the adjoining bone continues undisturbed A sclerosing action of infantile acute otitis media on the mastoid bone, as stipulated by Wittmaack, could not be found in an extensive study of histological material Due to the dissociation between the pneumatization curve and the bony cavity curve, the quantity of submucous connective tissue, instead of shrinking, increases Such a dissociation often disappears with the healing of acute otitis media, and after the reassociation of the two curves, pneumatization proceeds normally In some cases, probably due to a narrowed Eustachian tube, further attacks of acute otitis media occur and the dissociation continues over months and even years

Fig 5 is a vertical section through the tympanon of a 6-month old boy, suffering from an acute otitis media On the floor and mainly in the epitympanal region, submucous inflamed, connective tissue is present

In Fig 6 a vertical section through the mastoid process of the same case shows a dissociation of the two bone building processes Within the extensively preformed bone cavities, large quantities of connective tissue are still present below the inflamed mucosa Pneumatization is inhibited, while bone cavity formation still goes on

To control the further course of the dissociation we have studied 18 cases with adhesive processes due to infantile acute otitis In 11 cases no more dissociation and therefore no submucous connective tissue could be found, whereas in 7 cases a dissociation was still present

In Fig 7 we find a vertical section through the mastoid process of a girl, 4½ years of age, the bone cavity formation is extensive but the pneumatization is incomplete Furthermore, some residual adhesions in the large antrum and important amounts of submucous, loose, connective tissue are still present

Fig 8 is a vertical section through the tympanum of the same girl On the floor and in the anterior attic region submucous connective tissue is also still present

In Fig 9 we see the same region at higher magnification Prussak's space is filled with loose connective tissue, adhering to a slightly indrawn and thickened Shrapnell's membrane

It remains now to be seen what relation exists between the occurrence of a necrotizing infantile otitis media, initiating a chronic otitis media and the two cavity building processes? Necrotizing otitis media in infancy occurs irrespective of the situation within the middle ear space formation The resulting chronic otitis media with a persisting large central or marginal perforation of the tympanic membrane with severe inflammation of the mucous membrane within all middle ear spaces always [illegible]

[illegible] cells and bony sclerosis of the mastoid constitute a result rather than a cause of chronic otitis media we have studied the temporal bones of young persons undergoing radical operations We found an extensive to moderrate stage of cell formation in the mastoid in 55% of those between the ages of 5 and 10 years, and only in 12% between 21 and 25 years of age Thus, the older a patient afflicted with chronic otitis media is, the scantier are the cells in the mastoid where secondary sclerosis continues

On the basis of these surgical controls, we arrive at the conclusion—contrary to Wittmaack—that the relative lack of cells in sclerosis of the mastoid which is typical of chronic otitis media, does not constitute a cause but rather is a result of chronic suppuration in the middle ear, usually starting in early infancy

Before printing this rather long story in the form of a very expansive supplement to *Acta Otolaryngologica* I submitted the manuscript to Prof Otto Mayer, my first, admired teacher in otology He answered promptly "If K Wittmaack will read this paper, you better give up any academic aspirations " One year later (1937), my contribution was published I never heard a word from K Wittmaack Two years later, as a logical sequel, "Mittelohrraumentwicklung und Mittelohrentzündung" (Middle Ear Cavity Development and Inflammation of the Middle Ear) appeared and still not the smallest response from the addressed otologists

Stimulated by my late friend, E P Fowler, Jr, I went to Lempert's clinic from the 2nd January until mid March, 1939 From now on working with the rotating dental type burr instead of a mallet and a gouge and the use of Lempert's binocular loupe—soon replaced by Holmgren's otomicroscope—combined with sulphonamide and antibiotic therapy, the technique of ear surgery as well as the incidence and the course of ear infections was completely transformed within 15 years As mentioned, the incidence of necrotic otitis media in infants and, consequently, secondary acquired cholesteatoma diminished

Early in 1910, Bondy described a modified radical mastoid operation for the attic cholesteatoma with a Shrapnell perforation, an intact ossicle chain and an intact pars tensa Removing the superior meatal wall of the attic and taking down the bridge, fully exposed and exteriorized the cholesteatoma strictly localized in the attic Juers (1948) and Davis & Walsh (1950) detected the two basic principles of tympanoplasty, namely *sound protection of the round window*, and *sound pressure transformation for the oval window*

Moritz (1950) was the first to describe the use of pedicle flaps to construct a closed middle ear cavity in cases of chronic suppuration Thanks to this modified radical mastoidectomy the disagreable secretion out of the open Eustachian tube could be stopped in most cases In order to reduce the large cavity remaining after a radical mastoidectomy, T Palva and his co-workers introduced the "canal down" technique and mastoid obliteration

On the other hand, Zoellner (1951) and Wullstein (1952) developed their fascinating work on tympanoplasty, based on the aforementioned principles of sound protection of

the round window and reconstruction of sound pressure transformation for the oval window

Thanks to these successes, the surgical elimination of middle ear cholesteatomas was possible, mostly with preservation of the pre operative hearing or even with some amelioration Postoperative cleaning of the smaller cavity was still advisable every 6 months Cholesteatoma recurrences occurred in only 1–2% Further investigations and discussions concerning the pathogenesis of cholesteatoma were by now of only academic value and did not interest the majority of the practical minded ear surgeons

In 1958, Jansen (Germany) introduced a combined approach for all kinds of cholesteatomas the posterior tympanotomy (CAT, or intact canal wall operation) with the goal of eliminating the disease without deforming the normal anatomy and improving the hearing with the help of a simultaneous or staged tympanoplasty At first sight this was a very promising method and was subsequently popularized by W F House & J L Sheehy (1963) and by D A Corgill & L A Storrs (1967) among others in North America, whereas the European colleagues were rather sceptical In the U S A , only S Baron has warned (1967) that the use of these techniques might give rise to recurrences of the insidious disease

Fifteen years after the introduction of Jansen's closed or "canal up' operation, the first International Conference on Cholesteatoma took place in Iowa City (Iowa, May 26–29) Brian F McCabe, Jacob Sade and Maxwell Abramson, did a wonderful job, assembling 41 speakers from 26 countries Their contributions resulted in a unique book, *Cholesteatoma* of 468 pages, an interdisciplinary consideration of the etiology, basic mechanism, patho physiology and management of aural cholesteatoma (published in 1977 by Aesculapius Publishing Company Birmingham Albama)

Due to a motoring accident I was unable to assist at the meeting and I read the book only 2 months ago, with the greatest interest and very critically Retrospectively I wonder why, 2 years after the Iowa Meeting the same questions concerning closed versus open surgical treatment of the middle ear cholesteatoma had to be discussed at the First International Conference of the Politzer Society, at Davos in February 1978 I have agreed to read a paper on the pathogenesis of different kinds of cholesteatomas after reading in Harold Schuknecht's outstanding textbook *Pathology of the Ear* the introduction to the chapter "Keratoma' (Cholesteatoma) Otologists are in general agreement about the pathogenesis and histopathology of keratoma Simply stated, it is the accumulation of exfoliated keratin in the middle ear or other pneumatized areas of the temporal bone, arising from keratinizing epithelium that has invaded these areas from the external auditory canal "

I wondered why this experienced clinician was treating this still important dangerous disease like a stepchild Concerning Schuknecht's proposal to change the misnomer 'cholesteatoma' to 'keratoma', I looked up *Dorland s* [American] *Illustrated Medical Dictionary* where 'keratoma' is designated as a horny tumor In *Guttmann s Medical Dictionary* 'keratoma' is a special disease of the skin, characterized by a localized thickening of the stratum corneum

In the cholesteatomatous matrix, no tumour like localized thickening of the stratum corneum can be found On the other hand, the basal cells of the matrix develop, under conditions to be mentioned, capacities of growth never seen in a keratoma The two different diseases should therefore bear different names as in the past—cholesteatoma and keratoma

The report of the Politzer Society has not yet been published

The outstanding honorary guest of the Iowa Meeting, George Shambaugh, Jr, described how often he saw necrotizing otitis media and some developing secondary acquired cho

lesteatomas while he was on the staff of the Municipal Contagious-Disease Hospital in Chicago, whereas today "due to antibiotics and good nutrition the true secondary acquired cholesteatoma is almost unknown in our country", and Thane Cody (Rochester, Minnesota) in his introductory definition of cholesteatoma concluded "In a series of 483 ears with cholesteatomas that underwent mastoidectomy at the Mayo Clinic between January 1st, 1963 and December 31st, 1969, 483 (98%) were acquired cholesteatomas and 10 (2%) were congenital Traditionally the acquired cholesteatomas have been subdivided into primary acquired and secondary acquired cholesteatomas "

The primary type arises through the development of an attic defect and in Cody's series accounted for 389 (82%) of the acquired cholesteatomas The secondary type develops from marginal or central perforations of the tympanic membrane and accounted for 84 (18%) of the acquired cholesteatomas

Furthermore, the cause and development of these 84 secondary acquired cholesteatomas were left out of consideration and from then on, the subsequent Iowa Papers, with the exception of E L Derlacki, no longer mentioned secondary acquired cholesteatoma

Due to reduced numbers of foreign labourers in Switzerland during recent years, the number of secondary acquired cholesteatomas has diminished noticeably Nevertheless, I will discuss once more conventionally the secondary acquired cholesteatoma and the primary acquired cholesteatoma and even add two rare kinds—the traumatic cholesteatoma and the experimental cholesteatoma in guinea pigs Conceivably, after absorbing the findings presented at the Iowa Meeting American ear surgeons may be more interested in certain similarities to be found in all four kinds of cholesteatomas mentioned This might influence their techniques and improve their therapeutic results

I. Secondary Acquired Cholesteatoma

The typical onset and subsequent course of the disease is now described, based on four cases Two very rare possibilities of secondary acquired cholesteatomas, (i) following a subacute mastoiditis, breaking posterior-superiorly into the external meatus, and (ii) acute otitis media with perforation of Shrapnell's membrane, have been mentioned earlier (page 8)

The onset of a secondary acquired cholesteatoma is illustrated by the case of an 11-month old baby boy who died 14 days after the onset of osteomyelitis of the temporal bone, which had caused a left necrotizing otitis media on the third day

Histological examination of a transverse section shows a large upper marginal perforation of the ear drum (Fig 11) In the area of the perforation an active growth of basal cells of the adjoining epidermis of the external auditory canal can be seen One cone of basal cells enters the midde ear along the surface and, what is more important, a second cone of basal cells (matrix M) invades the submucous connective tissue (perimatrix P) and · not stop at the still intact epithelium of the middle ear This epithelium is now being undermined by the cones of basal cells

Here occurred apparently what D J Lim has seen with the help of the electron-microscope the break in the basilar membrane, presumably by physical disruption following severe inflammation The intense inflammatory reaction stimulates the basal cells to divide and proliferate On the other hand, the mesenchymal substratum, necessary for the growth of the basal cells, seems with the help of fibrial formation to guide the invasion of the basal papillary formations This relation between ingrowing basal cells and the mesenchymal substratum preoccupied me for quite some time Consequently D J Lim's findings are of the utmost importance in regard to the pathogenesis and the surgical elimination of the insidious cholesteatomatous disease

A further secondary acquired cholesteatoma can be examined in an 11-year-old boy whose cholesteatoma had started at the age of 8 years, due to a scarlatine necrotizing otitis media The boy died from meningitis, immediately after a radical mastoidectomy had been performed

Histological examination After growing actively through the inflamed, submucous connective tissue and through granulation tissue within the tympanum, the active growth of cholesteatomatous tubes continues within the pneumatized but severely inflamed mastoid cells

The cholesteatoma matrix undermines the mucosal epithelium, lifting it up and pushing it away as it advances (Fig 12) In the next section, the cholesteatoma breaks through the mucosa and masses of desquamated squamous epithelium are shed into the chronically infected cells (Fig 13) At the same time, the surrounding bony tissues respond to the chronic inflammatory stimulus by laying down new bone This secondary sclerosis eliminates many peripheral cells On reaching the bone, the active growth stops, the matrix does not attack the bone

However, in a second, more passive phase, the cholesteatoma sac continues to enlarge due to a persistent desquamation of horny lamellae from the thinned matrix at the expense of the surrounding sclerosed bone

In a third case, the progression of a secondary acquired cholesteatoma within a still incompletely pneumatized, large mastoid cell system can be seen A girl of 19½ years, who suffered from spinal tuberculosis, contracted at the age of 18 years a painful right sided otitis media, with severe deafness and inter-

mittent otorrhoea Otoscopy revealed a large superior marginal perforation of the drum, showing cholesteatomatous material within At the radical operation, a large cell formation was found within the mastoid bone The girl died of postoperative erysipelas

Histological examination of a horizontal section through the petrous temporal bone demonstrates high up in the attic a still present cholesteatoma within incompletely pneumatized cells Solid cones of basal cells are actively growing within the submucous connective tissue and the cellular bone responds to the chronic inflammation by formation of new bony lamellae within the inflamed connective tissue (Fig 14) At higher magnification (Fig 15), the active growth of the matrix proceeds within the inflamed connective tissue, while the secondary sclerosis takes place and reduces the same connective tissue

On the basis of these postoperative findings, it appears that the surgical treatment in the second and third case was not sufficiently radical Had the girl survived, the ear disease would have persisted within the incompletely pneumatized cells still present The large layers of submucous connective tissue are evidently a favorable substrate for the active growth of cholesteatomatous basal cell cones and tubes

The final stage of a secondary acquired cholesteatoma is illustrated by the fourth case, that of a 30-year old woman Since scarlet fever in early childhood she had had a foul smelling discharge from her right ear There was a large superior marginal tympanic perforation present with signs of a cholesteatoma A sclerosed mastoid was found at radical mastoidectomy The patient died of sepsis

Histological examination of a horizontal section across the petrous temporal bone (Fig 16) shows an expanded attic, lined by a cholesteatomatous matrix This matrix seems firmly but in some places irregularly fixed to the somewhat enlarged underlying promontorial bone The basal layer of the matrix is narrow and the individual basal cells have become flattened and spindle-shaped instead of their original cubical shape Reaching the bone, the active cholesteatomatous growth has stopped Again, the matrix has not attacked the bone

However, in a second, more passive phase, the cholesteatomatous material continues to enlarge due to a persistent desquamation of horny lamellae from the thinned matrix at the expense of the surrounding, more or less sclerosed bone

According to Abramson, Sade & McCabe, until 10 years ago, the most popular agent for the effect of cholesteatoma on bone was pressure "Although pressure has never been demonstrated in cholesteatoma " I wonder if the experienced ear surgeon Abramson has never found, within the cholesteatomatous masses, a denuded facial nerve, which instead of being round was transformed into a flat band due to the cholesteatomatous compression The single flattened nerve fibres might still be functioning, but they are often so fragile that the most careful cleaning will break some of these thinned fibres

Ten years ago I was not aware of the papers on "Cortical Bone Healing" (1969) from Stephan M Perren and his co-workers (Laboratory for Experimental Surgery, Swiss Research Institute, Davos, Switzerland) There was much confusion about the effect of different kinds of load (dynamic or static) on bone Perren et al could show that relatively small dynamic pressure changes can engender bone resorption These forces may be developed by a growing aneurysm for instance, or by a foreign body, anchored to bone undergoing passive movement The effect of static load is not the same Osteotomized cancellous bone, fixed under static pressure, will quickly unite by new bone formation Adapted to our problem The cholesteatomatous material increases irregularly and, due to humidity or secondary infection, small dynamic pressures should occur and produce bone resorption Normally the middle ear cavity

and the pneumatic cell systems are lined by a very thin mucoperiosteum (Fig 17) The basal membrane of the flat epithelial cells lies directly on the delicate periosteum

Jens Thomsen and his co workers showed with the help of light microscopy and electron microscopy that in chronic otitis media both without and with cholesteatoma, after the destruction of the thin mucoperiosteum, the bone was always covered by a granulation tissue Capillary proliferation is the chief finding in the resorbing margin of the bone and the dominating cell in the eroded marginal zone is a mononuclear, histiocyte like cell with dense cytoplasmatic bodies of lysosomal character

Maxwell Abramson, M D and Cheng-Chung Huang, Ph D were seeking a specific collagen dissolving enzyme, to explain bone resorption A single cholesteatoma weighing 100 mg could dissolve as much as 400 μg of collagen in 4 days in vitro Whilst looking for the source of this enzyme, no sign of collagenase was found in the epithelium But the enzyme has been found in fibroblasts, in certain monocytes, and in endothelial cells of capillary buds within subepithelial connective tissue Both capillary buds and monocytes are increased in chronic inflammatory states, producing fistulas in guinea pigs, using talc granulomas and covering skin flaps The resence of epithelium greatly increased the incidence of bone resorption in these animal experiments

Now, bone resorption cannot be understood by simply studying collagen breakdowns The demineralization process is at least as important and is probably a necessary primary step before collagen breakdown can occur Bruce J Gantz and collaborators studied in guinea pigs the activity of prostaglandin, one of the mediators responsible for producing demineralization of bone As prostaglandin is present in chronic inflammatory tissue, it may play an active role in localized bone resorption in chronic middle ear disease

These identical microscopic findings lead macroscopically to very differing results Why, in cases of chronic otitis media *without* cholesteatoma, does this granulation tissue, capable of bone resorption, not visibly enlarge the middle ear cavities and never produce labyrinthine fistulas, whereas in cases of chronic otitis media *with* cholesteatoma the same granulation tissue, squeezed between the matrix and the bone, produces the wellknown extensive and dangerous bone resorption? Even the very hard bone of the inner ear capsule does not resist Also in our fourth case, a fistula was found in the horizontal semicircular canal (Fig 18) Granulation tissue with many capillaries below the matrix is resorbing the hard canal bone (Fig 19) Why does the bone resorption stop in cases of incomplete elimination of the matrix (as in the case described by George Shambaugh) as long as the still desquamating epithelial layers are regularly removed from the large open cavity? Until we get better answers to these questions the assumption seems likely that the varying pressure (dynamic load) within the dead cholesteatomatous material activates the bone resorbing functions of the bone covering granulation tissue

In this fourth, typical case of a long standing secondary acquired cholesteatoma radical mastoidectomy has completely eliminated the disease, leaving only the matrix over the fistula as a protective cover Dysfunctioning of the Eustachian tube is not involved in the pathogenesis of secondary acquired cholesteatoma

Surgical treatment of the secondary acquired cholesteatoma depends on the age of the patient In adult patients seen in our country today, the petrous bone is sclerotic and poor in cells due to the long standing chronic otitis media The sound conduction system is mostly destroyed Tympanoplastic procedures are not very promising In these cases, Palva's canaldown and mastoid obliteration technique has regularly been used at the Zurich Clinic In a large number of similar cases we had (roughly judged) no more than 2% of recurrent cholesteatomas

II. Primary Acquired Cholesteatoma

The primary acquired cholesteatoma originates, as we will try to prove, prior to the perforation of Shrapnell s membrane and consequently before the onset of chronic otitis media On the other hand, the majority of the American participants at the Iowa Meeting considered Habermann's and Bezolds's Eustachian tube dysfunction as the basic abnormality leading to the attic retraction cholesteatoma, the conception of George Shambaugh, Jr Accordingly, Th Cody defines a cholesteatoma as "stratified squamous epithelium, trapped and growing in foreign sites within the temporal bone, resulting in the production of a progressively expanding tumor mass, consisting of new growth of epithelium, various stages of degenerating epithelium, abundant keratin and usually associated with cholesterol and inflammatory cells' Somewhat later, Dr Cody adds "The definition of acquired cholesteatoma should include its association with an attic defect, the presence of a very sclerotic mastoid process and various degrees of Eustachian tube dysfunction' The 18% of acquired cholesteatomas in Cody's statistics with large or total upper marginal perforations of the tympanic membrane, which occurred independently of Eustachian tube dysfunction, are—as mentioned—quite forgotten The amount of mastoid bone sclerosis depends, as has been shown, on the duration of a chronic otitis media with or without cholesteatoma Accordingly, in today s statistics, a sclerotic mastoid process is found in 84% of cases with cholesteatomas, and in 72% of patients with chronic infection but without cholesteatoma

Only Charles D Bluestone and his collaborators (1977) measured the function of the Eustachian tube with an elaborate inflation/deflation technique in different groups with and without middle ear cholesteatoma Five patients with cholesteatoma (3 in the posterosuperior region, 2 in the pars flaccida) had evidence of functional obstruction of the Eustachian tube In addition, the five patients with cholesteatoma had tympanometric evidence that the tympanic membrane was intact What appeared to be a perforation under the operating microscope, was actually a deep sac, not open to the rest of the middle ear cavity

The question arises, were these sacs delineated by sucked in epidermis or by an actively growing cholesteatomatous matrix? Until further pressure measurements and histological controls are made, the causative importance of Eustachian tube dysfunction still seems doubtful

The main interest especially of the younger very active otologists, was naturally concentrated on the long term results, obtained by 10 experienced ear surgeons, 4 by using the classical or modified radical mastoidectomy and 6 using mainly the *new canal up technique* for the elimination of acquired cholesteatoma

SURGERY CLASSIFICATION

Group A (Radical technique)

I *T Palva* and his collaborators (1977) operated on 200 cholesteatomas during 2 years, performing a typical radical mastoidectomy, reconstructing the ear canal with a fascial graft posterior to the canal skin and occluding the mastoid cavity with a musculo-periosteal flap Recurring cholesteatomas occurred during a control period over 4–6 years in 2% In 10 cases, a postoperative retraction developed These ears were not self cleaning and were prone to infections, if cleaning was per

formed at intervals longer than 3–4 months No retraction pockets with cholesteatoma formation occurred

2 *Harold F Schuknecht* (1977) used for ears with extensive involvement of the epitympanon or mastoid the canal down technique in association with Type III or Type IV tympanoplasty and fascial muscle obliteration of the epitympanon and mastoid In 347 cases cholesteatoma, postoperative cholesteatomas occurred in 25 (2 7%) In 70, Type IV tympanomastoidectomies, performed over an 11 year period, cholesteatomas were present in 63% and postoperative recurrences were noted in 4 28%

3 *Shirley H Baron* (1977), performed 99 cholesteatoma operations with 3 or more years' follow-up 76 modified radical, 21 radical, 1 intact canal wall procedure, and 1 transcanal attico antrotomy Recurrent cholesteatomas were noted in 8% No retraction pockets are mentioned

4 *Richard J Bellucci* (1977) did a radical mastoidectomy on 81 extensive cholesteatomas, recurrent cholesteatomas occurred after 2 years in 3% The majority of 122 cases required a Type II or III reconstructive tympanoplasty 21 of these patients (or 23%) had a recurrence of the cholesteatoma

roup B (Canal up technique)

Claus Jansen (Germany) In 441 cases of cholesteatoma (5 with fistulae in the semi circular system) after posterior tympanotomy, followed from 1958 until 1969, a recurrent cholesteatoma occurred in only 3%

2 *Michael E Glasscock* reports (1977) 154 cases of cholesteatoma, managed by an intact wall tympanoplasty He observed recurrent disease in 14%

3 *William K Wright* (1977) has operated with a canal-up technique on 52 cholesteatomas and on re-exploring all these cases he found regrowth cholesteatoma in 15 4%

4 *Gordon L Smyth* (Ireland) performed over a 13-year period closed operations in 532 cholesteatomatous ears Recurrent cholesteatomas occurred in 7%, residual cholesteatoma in 23% "It follows that the place of once only CAT in the routine management of chronic otitis media with cholesteatoma requires further assessment "

5. *D T R Cody* reports (1977) the results of 171 intact wall mastoidectomies, performed for acquired cholesteatoma, within 7 years 60 (35%) failed because of recurrent or residual cholesteatoma 68% of the cholesteatoma failures were due to recurrent cholesteatomas, 20% of the mastoidectomies (34 ears) failed because of precholesteatoma, which is defined as the formation of an attic or facial recess retraction pocket 19 of the 34 pre cholesteatoma failures were also associated with chronic infaction *"Pre-cholesteatoma* for retraction pockets has proved to be an appropriate term because the recurrent cholesteatoma first develops a retraction pocket and then forms a cholesteatoma"

Thane A Cody and *William F Taylor* (1977) analysed in an excellent statistical work the long term results after 423 mastoidectomies for acquired cholesteatomas The mastoidectomies were divided placed into (*a*) an open cavity group (37 radical mastoidectomies and 135 modified radical mastoidectomies), (*b*) an obliterated cavity group, and (*c*) the intact canal wall group

The overall failure rate was 2 3 per 1000 months at risk for group (*a*), 3 6 for group (*b*), and 13 0 for group (*c*) "When successful mastoidectomy in the three groups was compared, open cavity mastoidectomy required only slightly more care than did those in the other two groups *Open cavity mastoidectomy should be primarily used in the treatment of acquired cholesteatoma*

6 *D F A Austin* (1977) introduces his article with an excellent definition of cholesteatoma "The keratinizing epithelial pocket of cholesteatoma has three characteristics Its invasive potential, its progressive destruction of bone and, most important, its tendency to recur "

Within 5 years, Dr Austin performed 124

cholesteatoma operations In the canal preservation group (60), persistent disease occurred in 25% following retraction pockets, 7% due to other complications "Neither negative pressure nor inflammation seem to be factors involved in retraction, since neither was observed in this group of patients Traction does not seem to be causative in pocket formation, since scarring was not evident of revisional surgery nor did silicone sheeting prevent such recurrence "

Although several contributors have seen the development of a cholesteatoma out of a retraction pocket, only Th Cody (*vide infra*) and D F Austin (Chicago) described the history of such an event According to the latter author, a retraction pocket will be seen to develop usually between 18 and 30 months postoperatively Its appearance is usually unheralded, although some patients will note a slight change in hearing If the pocket is very developed some granulation may appear at the postero superior margin secondary to erosion, and secretion will now be present In most patients the pocket will acquire all of the characteristics of the original disease growth bone destruction and—if operated on by the same technique—a second recurrence If untreated the growing destructive pocket will cause loss of the thinned posterior canal wall resulting in an unstable mastoid cavity The best treatment of the retraction pocket is now the radical mastoidectomy with mastoid obliteration

As mentioned T H Cody also describes the development of a cholesteatoma out of a retraction pocket

A 49 year-old woman was first seen in March 1967 She had a lifelong purulent discharge from her left ear A polypus was noted growing through a large central perforation in the tympanic membrane Roentgenograms revealed a very sclerotic mastoid process On March 8 1967 a left simple mastoidectomy with opening of the facial recess revealed haemorrhagic granulation tissue throughout the tympanic cavity epitympanon and antrum The perforation of the tympanic membrane was repaired with a temporalis fascia graft The ear healed satisfactorily and on August 18 1967 a transcanal tympanoplasty was performed to reconstruct the ossicular chain The ear again healed uneventfully On April 21 1972 the patient returned because of a throat complaint She had no trouble with her left ear Examination revealed a large postero-superior retraction pocket of the left tympanic membrane extending into the facial recess but no cholesteatoma In June 1974 the patient began to have postural unsteadiness and developed vertigo when she pressed on her left ear In October 1974 examination revealed a purulent discharge in the left ear and extensive cholesteatoma in the mastoid process and findings consistent with a fistula of the horizontal semicircular canal A left modified radical mastoidectomy on October 17 1974 revealed a mastoid packed with cholesteatoma which had obviously arisen from a retraction pocket that had formed through the previously open recess

Evidently this iatrogenic primary acquired cholesteatoma developed over several years behind or out of an intact retraction pocket producing even a labyrinthine fistula before breaking through the retraction pocket into the external meatus A Eustachian tube dysfunction may have favoured the formation of a retraction pocket but cannot be the cause of the cholesteatoma formation

Comparing the postoperative results of conventional canal down techniques with the results after a canal up intervention the failures in the latter group are distinctly more numerous Retraction pockets following canal up operations and later developing into cholesteatomas are the greatest cause of these failures And again, based on impressions rather than measurements of intratympanic pressures, Eustachian tube dysfunction is considered to be the cause of retraction pocket formation W Wright, who reported a 12% incidence for significant retraction pockets, does not wait for them to form cholesteatomas He removes these " pre cholesteatoma" surgically Unfortunately, up to now, as far as I known none of the ear surgeons has used this unique opportunity to study histologically the removed retracted pocket As in an experiment, the appearance of the cholesteatomatous matrix behind or out of the epithelium of the retracted pocket should be traceable I have no personal experience with canal up mastoidectomies, but could an attic or facial retraction pocket not develop within 1–2 years after a canal up mastoidectomy, due to the normal pressure changes within the middle ear cavity? Every tympanoplasty, usi

A I I OII

to close a defect of the tympanic membrane, is rebuilt within 1–2 years into a flaccid scar tissue, covered outside with a thin layer of *tympanic epithelium, and mucosa on the tympanic side* Such a scar may be mobile, free to move in response to pressure changes within the tympanon Lacking the elasticity of the pars tensa, a mobile pocket known to every otologist occurs If such a retraction pocket adheres and becomes attached to mesenchymal structures—for example granulation tissue in the tympanic cavity—the predisposing factors for the development of a cholesteatoma *are fulfilled*

George T Nager, who was for 2½ years my collaborator at the Zurich University Clinic, has also shown with the help of beautiful designs the development of Shrapnell cholesteatomas According to Nager "The two basic mechanisms involved in the pathogenesis of the attic or Shrapnell's cholesteatoma re *(1) retration of Shrapnell's membrane* (attic retraction), and (2) active epithelial im*migration, each with the propensity of forming* an attic cholesteatoma "

"The predisposing factors for papillary proliferation and immigration of squamous epithelium into the middle ear comprise (1) an inherent growth potential of the basal cell layer of the epidermis, covering the outer surface f the drum, (2) the submucosal connective issue in the middle ear, (3) hypocellular pneumatization "

"The provocative impulse for active epithelial proliferation and immigration is an inflam*matory process, generally in the middle ear* '

Most otologists accept today Habermann's immigration theory In a beautifully illustrated *article, Jacob Sadé (Israel) tests the possibility* of metaplasia within the attic mucosa as the *cause of attic* cholesteatoma But the electron microscopical findings of Georges Bremond & Jacques Magnan (France) of Langerhans cells and Merkel's cells within the cholesteatomatous matrix makes Dr Sadé's metaplasia theory very untenable 'Langerhans and Merkel's cells are seen in the skin, but do not normally exist in the middle ear mucosa Metaplasia *of the middle ear mucosa can give* epidermoid squamous epithelium It is hard to *believe, that this metaplasia results in the formation of such particular cells as Langer*hans' and Merkel's cells Their presence in cholesteatomas seems *to provide a good argu*ment in favour of the migratory theory in this lesion "

David J Lim, and his co workers (1977) have studied with the help of a phase contrast microscope and a transmission electron microscope (Philips 300) 75 aural cholesteatoma specimens From this, in my opinion most important contribution, I quote parts of the chapter on the "Advancing Front and Cyst or Pearl Formation"

"A majority of the cholesteatomas we examined had a cystic form but a few of them were in a typical pearl form The former type of cholesteatoma appears to be the *result either of the ingrowth of epidermis from the tym*panic membrane perforation mainly from Shrapnell's membrane or of total perforation of the whole drum Some of them appeared to originate from an indrawn tympanic membrane (retraction pocket) of which the mucous layer has adhered to the middle ear connective tissue by inflammation

When the cholesteatoma maintains a clean pearl formation the matrix and perimatrix are usually very *thin due to the distension of the sac by the collection* of keratin matter in it However when the cholesteatoma is in a cystic form in direct contact with inflamed mucosal [illegible] format on

[illegible]

matrix Importantly where the localized inflammatory reaction takes place the papillary projection of the dermis is evident In some instances the cholesteatoma matrix *with or without a papillary projection may form a pearl* similar in mechanism to that of Rüedi's (1959) experimental cholesteatoma It appears that an important prerequisite for cholesteatoma invasion or pearl or cyst formation of acquired cholesteatoma is an inflammatory reaction

When the advancing front of the epidermis is examined there are numerous fine fibrils attached to the basal cells serving as a guide Examples of several dif*ferent modes of epidermal advancement can be found in* temporal bone sections where varying advancing fronts can be studied in a serial manner Depending on the direction of the guiding fibrils the advancing epidermal basal [illegible] he muco- [illegible] Vhen [illegible] rmis [illegible] ation

Electron microscopic findings of such advancing fronts

confirm the light microscopic observations and further show that the basal lamina is disrupted and the pseudopodial extension of the migrating cells can be observed in contact with the fine fibrils Migrating or newly migrated epidermal cells lack desmosomes—structures binding cells together—perhaps limiting their mobility

These findings are particularly important for both theoretical and practical consideration This finding would imply that the behavior of the migrating epidermis is dictated by the fibril formation and subsequent shrinkage as a result of a presumed local inflammation not as a result of presumed invasive nature of the epidermal cells in the cholesteatoma as suggested earlier It can be hypothesized that the extent of invasive behavior may be determined by the microchemical environment particularly the contraction of plasma surrounding the migrating front This concept is also in line with the common clinical experience that a quiescent cholesteatoma can suddenly become active with aural infection A still unanswered question is the nature of the underlying inflammtion

Considering the great number of mostly excellent contributions, the conclusions of the whole meeting from Brian F McCabe, Jacob Sade and Maxwell Abramson are very cautious "Cholesteatoma is not simply skin at the wrong place but a three dimensional epidermoid structure, exhibiting independent growth displacing or replacing middle ear mucosa, resorbing underlying bone, and tending to recur after removal The subepithelial inflammatory connective tissue promotes bone resorption through decalcification, followed by sequential enzymatic breakdown of bone matrix While osteoclasts are capable of resorbing bone under normal conditions, the evidence is that localized bone resorption can occur from inflammatory connective tissue the action of cells turned on by inflammation This inflammatory connective tissue while always present under the epithelium of cholesteatoma, is also present in chronic otitis media without cholesteatoma No evidence was evinced that stratified squamous epithelium in itself had any destructive or nocuous properties "

Ten experienced otologic surgeons presented their longest term results available on different techniques, especially including closed cavity' techniques "There is evidence that we may be in some state of regression from the enthusiastic acceptance of the 'canal-up' mastoidectomy of the last decade "

The two most noteworthy features of this part of the conference were (*a*) there is a definite need for longer follow ups in these newer operations for adequate evaluation, and (*b*) at least a few surgeons voiced willingness to give them up

After this rather long comment on the papers read at the Iowa Meeting, I might add some clinical and histopathological findings, concerning the pathogenesis of primary acquired cholesteatoma, traumatic cholesteatoma and experimental cholesteatoma in the guinea pig

Collected during 40 years of E N T activity and publishing in different medical journals, these findings—repetitions of my own writing and reprints of published pictures—were unavoidable

PRIMARY ACQUIRED CHOLESTEATOMA

The supporters of the immigration theory eagerly search for the origin of the special energy of growth in the epidermis of the external auditory meatus, adjoining the tympanic membrane superiorly

We have examined the epidermis of the external auditory meatus of embryos, newborns and infants which had no ear pathology (53 ears, ranging in age from fifth fetal month up to 10 years) Our histological results were surprising all healthy embryos, newborns and infants show an increased growth in the sense of hyperkeratosis and acanthosis in a well defined section of the epidermis of the external auditory meatus, adjoining the tympanic membrane anteriorly and superiorly (Fig 20) In 7 out of 21 cases of cholesteatoma, Eigler (1951) has succeeded in demonstrating atypical growth in epidermis of the external auditory meatus, which showed no signs of inflammation and was also associated with formation of epithelioid cysts

Clinical observations

On examining 40 histological specimens of infantile cases of acute otitis media without perforation of the tympanic membrane, we found in every case more or less pronounced evidence of hyperkeratosis and acanthosis in the epidermis of the external auditory canal, adjoining the tympanic membrane superiorly In Fig 21 one can see in a vertical section through the left external auditory canal and Sharpnell s membrane of an 11-month old girl, suffering from acute otitis media, solid cones of basal cells growing actively into the con nective tissue, and filling Prussak's space Fig 22 shows the solid cone of basal cells at higher magnification Matrix (M) into the Perimatrix (P)

In the next case, of a 68 year-old man who suffered from a dry, recurrent, desquamating eczema of his right ear and who died from a heart attack, the growth of a cone of basal cells (M) within inflamed connective tissue (P) behind Shrapnell's membrane is shown in Fig 23 Sooner or later this cone will split up into a cholesteatomatous tube

In the next case, a boy 6½ years of age, had suffered since the age of 5 from a suppuration of the middle ear that was at times fetid At this time, the attending specialist found a perforation of Shrapnell s membrane and several holesteatomatous nodules and therefore recommended radical operation Only a short time later, the discharge from the ear ceased spontaneously and the perforation of Shrap nell's membrane healed, leaving only a small scar Only a moderate conduction deafness and a shaded mastoid remained, as seen in the X-ray film Despite this spontaneous healing the boy was operated on on the tentative diagnosis of cholesteatoma behind an intact tympanic membrane The operative findings revealed a mastoid with extensive cells, con taining abundant granulation tissue There was a cholesteatoma in the antrum running from the aditus along the tegmen and towards the angle of sinus and dura Behind the *intact* membrane of Shrapnell there was an addi tional nodule of the cholesteatoma approxi mately the size of a bean, that originated from the inner surface of Shrapnell's membrane and developed towards the tympanum proper and tube While carefully removing the chole steatoma, its origin at the inner surface of Shrapnell s membrane was distinctly visu alized with the oto microscope The narrow stem of the matrix, rooting in Shrapnell s membrane, caused a rupture of this membrane when severence was attempted In this case, the cholesteatoma has developed primarily from the initially intact Shrapnell's membrane

On the basis of this history, the assumption may be made with the greatest possibility that in this case a cholesteatoma developed pri marily from the initially intact membrane of Shrapnell in the manner we described above Eventually the congested epithelial masses perforated the weakened point of origin, and infected material of the cholesteatoma was discharged for some time into the external auditory meatus through the perforation in Shrapnell's membrane The congested mass es, having been discharged and the inflam matory reaction having subsided, the perforation in Shrapnell s membrane closed spon taneously, but the cholesteatoma continued to grow behind the now healed tympanic mem brane

These clinical observations of the devel opment of a cholesteatoma behind the intact tympanic membrane complement, in our opinion, the histological findings, according to which cholesteatomas of Shrapnell s mem brane are caused by an inflammatory immigra tion of epidermis of the external auditory meatus

Therefore our investigations confirm every observation and conclusion of Lange, concerning the onset of the primary acquired cholesteatoma

Due to the continuous desquamation of epithelial layers within a narrow space, the accumulated cholesteatomatous material will in most cases sooner or later break through Shrapnell s membrane, discharging some of

the cholesteatomatous mass into the external auditory canal and leaving a small perforation in the Shrapnell membrane

The break in the membrane and the small discharge of cholesteatomatous material occurs mostly unobserved by the patient Meanwhile the further proliferation of the actively growing cholesteatoma into the middle ear cavity continues

Today, Lange's findings and view on the onset of primary acquired cholesteatomas are accepted by most otologists—at least in the German-speaking countries

The fact that a primary acquired cholesteatoma can grow in clinically silence over many years, and from now on independent of a disfunction of the Eustachian tube, into the antrum and the mastoid cells, is well known But as far as I can ascertain, this intriguing fact is not explained in the literature

Based on the just demonstrated dissociation of the two cavity-building processes, due to infantile acute otitis media, layers of submucous loose connective tissue are still present in some infants within the middle ear cavity, while the preformed cells in the mastoid bone, due to the retarded pneumatization, are completely filled by connective tissue Therefore, in my opinion, a primary acquired cholesteatoma develops under these special conditions, whereas as mentioned, the secondary acquired cholesteatoma starts independent of middle ear space formation In these cases the basal cells need for growth either submucous connective tissue or granulation tissue, matrix and perimatrix The cones of a primary acquired cholesteatoma (matrix) emanating from Prussak s space, instead of entering the tympanon, grow actively and even stimulated within the still present, non-inflamed, submucous connective tissue (perimatrix), crossing the tympanon without breaking into it From the incompletely pneumatized antrum the cholesteatomatous tubes grow finger like further on into the not pneumatized cells As long as no secondary infection of the desquamated epithelial lamellae occurs, no reactive sclerosis of the bone will take place, whereas, as seen in the third case of secondary acquired cholesteatoma, an infection of the cholesteatomatous lamellae will start new bone formation within the layers of connective tissue

Subsequently, the further growth of the infected primary acquired cholesteatoma will first in an active, and later in a more passive phase, continue identically to the progress of a secondary acquired cholesteatoma observed within an incompletely pneumatized cell system But due to the relatively mild and circumscribed inflammatory irritation, the secondary sclerosis is less pronounced and the endocranium more endangered

In support of these statements, the clinical observations and histological findings in a further case of primary acquired cholestea toma are described A 21-year-old man devel oped a foul-smelling discharge from his left ear, together with a left hearing loss and intermittent earache In his 23rd year, there was a sudden increase in the severity of the earache, with profuse otorrhea and fever, for which an otologist referred him to our clinic, where, a Shrapnell s perforation was discovered with discharging masses of cholesteatomatous material Radical operation on the left ear revealed a partially sclerosed mastoid with a large cholesteatoma which had eroded the tegment tympani and was lying directly up against the dura The patient succumed to otogenic meningitis with a temporal abscess

The histology of a vertical section through the middle ear in Fig 24 reveals a typical Shrapnell's perforation, with the cholestea toma can be seen here actively growing within the connective tissue (Fig 25), whereas nearby new bone formation reduces the connective tissue

In Fig 26, the transition from the primary active phase of cholesteatoma growth to the secondary more passive phase, where the matrix contacts, bone is represented. Between the thinned matrix and bone, the lighter zone of granulation tissue is visible.

The radical mastoidectomy in this case was done not sufficiently radically and this is the great danger in all cases of primarily acquired cholesteatomas

Finally, in a small but much discussed group, the cholesteatoma is occasionally found behind an intact tympanic membrane due to a sudden conductive deafness, a facial palsy, vertigo, or signs of intracranial complications The intact Shrapnell s membrane is in most cases slightly indrawn or thickened by scar formation Between 1963 and 1965 at the Zurich University Clinic, 9 Shrapnell cholesteatomas behind an intact tympanic membrane have been diagnosed and operated on Four patients (20, 29 and 34 years of age) suffered since their early youth from recurrent acute otitis media, sometimes with fetid *secretions and a serious sound conductive* deafness In one case, facial palsy occurred, another patient had attacks of dizziness In all 4 cases, behind the intact tympanic membrane, a large cholesteatoma was found, originating from Shrapnell's membrane and destroying the sound conductive ossicles In one case, the facial nerve was compressed In the patient suffering from vertigo, a fistula of the horizontal semicircular canal was found In all 4 cases radical mastoidectomy with tympanoplasty was performed, but failed to ameliote the deafness The 5 further Shrapnell cholesteatoma behind an intact tympanic membrane were discovered in 4 small children—*in one case bilaterally In two ears,* the ossicle chain was already interrupted *The cholesteatoma had* reached the antrum, a conservative radical mastoidectomy was therefore necessary In three ears the cholesteatoma, originating from the intact Sharpnell' membrane, had not harmed the ossicle chain and could be eliminated with an atticotomy The normal hearing of these 3 children did not deteriorate postoperatively

Some of the published congenitive cholesteatomas might also have been primarily acquired cholesteatomas, originating from Shrapnell s membrane without secondarily breaking into the external meatal canal (Fig 27) As long as no secondary infection of the desquamated material occurs, active growth within the submucous *connective tissue of* incompletely pneumatized cells continues without a sclerotic reaction of the bone, and as already described, the cholesteatoma will, due to the second passive phase of growth, slowly destroy the adjacent bone

Finally in the case of a 23 year-old man with a perforation of Shrapnell s membrane, filled with cholesteatomatous material, who succumbed to otogenous meningitis and brain abscess immediately after radical mastoidectomy, the still active growth of the cholesteatomatous matrix (Fig 28) could be demonstrated histologically Even the radical mastoidectomy had not eliminated all active *cones of matrix within the connective tissue,* still filling performed bony cells

Therefore, in many primary acquired cholesteatomas, canal up surgery will not completely eliminate this insidious disease Recurrences will still develop slowly and destroy hearing gains achieved by technically excellent tympanoplasties

The belief that the active growth of a primary acquired cholesteatoma takes place mostly within layers of submucous connective tissue due to a dissociation of the two cavity-building mesenchymal factors, can be criticized because of a gap in our histological argument Up to now, the presumed active *submucous and therefore clinically silent* growth across the tympanum has not been *histologically demonstrated This criticism* will only be countered by histological examination of an *untreated* primary acquired cholesteatoma behind an intact tympanic membrane, which has not yet been possible

TRAUMATIC CHOLESTEATOMA

Meanwhile, Escher's discovery of a rare form of a *Traumatic Cholesteatoma* might support our ideas

After a bicycle accident, a 29 year-old man observed some bleeding from his right ear

and noticeable and persistent hearing loss in this ear 15 years later, a slowly increasing facial palsy started on the right side, followed 2 months later by a small amount of fetid secretion from the right ear One year later, the facial palsy was complete Escher found a marked conductive deafness and no reaction of the right vestibular apparatus to caloric stimulation Otoscopy showed an intact tympanic membrane and a small step in the sulcus, bordering Shrapnell's membrane Some fetid cholesteatomatous material was found within a minute perforation

In the Stenvers radiological picture, a defect of the superior border of the pyramid was visible, whereas the mastoid process was normally pneumatized

The operation showed an old fracture line passing through the squama temporalis, ending within the external auditory canal in the region of Shrapnell's membrane At this place, a cholesteatoma originated directly out of the fracture line and had destroyed the tegmen tympani and the superior semicircular canal, and reaching the dura mater, had eroded the superior border of the pyramid An exposed compressed facial nerve was found It is worth noting that the cholesteatoma did not follow the fracture line and bypassed the normally pneumatized mastoid process

Reconstructing the origin and course of Escher's rare traumatic cholesteatoma, the following concept seems probable in this case Sixteen years ago a fracture ending within the right external canal occurred with tearing of the meatal skin in the region of Shrapnell's membrane Some squamous epithelial particles must have become dislocated within the bone fissure The inflammatory irritation of the healing fracture caused an active growth of the very potent epithelial basal cells Within the fresh granulation tissue filling the bony fissure, a matrix and a cholesteatomatous tube was formed behind the healing tear in the meatal skin On the other hand, the simultaneously healing bony fissure was closing because of new bone formation Therefore, on reaching the bone and in default of further connective substratum, the active growth of the matrix stopped Instead of the delicate endosteum, a small zone of granulation tissue developed between the thinned matrix and the bone Due to the continual but irregular desquamation of epithelial lamellae of the matrix, pressure changes occurred within the cholesteatomous sac, stimulating the bone-resorptive faculties of the granulation tissue

In a second, more passive phase of growth, the cholesteatoma sac enlarged slowly, following the bone resorption along the route described

This traumatic cholesteatoma started in a way similar to a primary acquired cholesteatoma, also independent of a dysfunction of the Eustachian tube Within the completely pneumatized middle ear cavities, there were no submucous layers of connective tissue present which could have favoured the active process of the primary acquired cholesteatoma on its typical way through the tympanon into the mastoid cells

Without this mesenchymal substratum, the passive extension of the cholesteatomatous sac grew silently on over a period of 15 years at the expense of the surrounding bone, independent of the middle ear spaces

The surgical treatment had in this fully pneumatized and uninfected case only to follow and eliminate the flattened matrix

EXPERIMENTAL CHOLESTEATOMA IN GUINEA PIGS

Typical cholesteatomas in animals have been produced by the application to the external auditory meatus of cold or hot tar, benzpyrene, croton oil or olive oil or by injection of these substances into the middle ear (Berberich, 1927, Hoshiya, 1935, *Ohta, 1940*, Schroer, 1957) The skin of the deep meatus proliferates in these experiments and grows into the middle ear through a myringotomy opening or spontaneous perforation No signs metaplasia of the mucosa of the middle ear

were found on any occasion In this connection, special mention must be made of the brilliant experiments performed by J Friedmann (1955) who infected the middle ear in guineapigs with *Pseudomonas pyocyanea* and with *Streptococcus pneumonicus* He then found that "the stratified squamous epithelium from the external auditory meatus or from the tympanic membrane immigrated through the perforated tympanic membrane into the infected bulla of the guinea pig, reproducing here a typical aural cholesteatoma" J Friedmann's experimental findings fit in very well with J Habermann's classical observations in necrotizing otitis media of infants

A review of the literature shows that a tympanic perforation was present in all the experimental cases of cholesteatoma The cholesteatoma developing behind an intact tympanic membrane, which is of particular interest to us, has not yet been reproduced experimentally

In order to test this possiblity, we (Ruedi & Spoendlin) have opened the aural bulla in guinea pigs and affixed a mixture of talc and fibrin in several places to the internal surface of the intact tympanic membrane This caused a mild foreign body reaction and, in several animals granulation tissue developed between the drum and the internal wall of the middle ear (Fig 29) After 15–20 days there occurs in some animals an active invasion of the basal cells from the intact tympanic membrane through a break of the basal membrane into the newly formed granulation tissue The invading columns of basal cells divide into branches and the desquamated stratified squamous epithelium forms typical cholesteatomatous masses Thus a mild inflammatory reaction *resulting from chronic irritation may pro-*duce a cholesteatoma behind an intact tympanic membrane in the guinea pig This renders the propostion, that human aural cholesteatomas behave in much the same way, all the more likely

TREATMENT

To achieve success in the treatment of every case of cholesteatoma, one must strive for the complete elimination of the entire cholesteatomatous matrix and also of the perimatrix This goal can be reached by means of atticotomy (Bondy) when, exceptionally, the onset of a cholesteatoma formation out of an intact Shrapnell's membrane is suspected

As soon as the cholesteatomatous tubes have reached the posterior wall of the tympanic cavity, only radical mastoidectomy, modfified by Palva's technique, can make the necessary cleaning of the tympanic cleft and the communicating cell system possible The high recurrence rate after a canal up posterior-*superior tympanotomy is in my opinion attri-*butable to incomplete elimination of matrix and especially of perimatrix, rather than to disfunction of the Eustachian tube

The incidence of attic cholesteatoma can be diminished on the one hand by an intensified instruction of general practitioners and pediatricians on the pathogenic importance of recurrent acute epitympanic otitis media in infants for cholesteatoma formation

On the other hand, by means of preventive surgery in suspect cases, a routine surgical control of Prussak's space with the help of an endaural skin flap is achieved and eventually clearing of this region and plugging of Prussak's space to separate basal cells (future matrix) from connective tissue or granulation tissue (future perimatrix) should reduce the incidence of attic cholesteatomas

Résumé

O Steurer W Albrecht et M Schwarz ayant confirmé les donnes de Manasse et Lange concernant la genese du cholestéatome moi meme ayant constaté des erreurs dans la doctrine de Wittmaack la plupart des otologues lisants l allemands a renonce à la theorie de retraction de Bezold

L influence des infections sur le developpement des cavités de l oreille moyenne fut examinee D apres ces resultats combinés avec les nouvelles observations de David I Lim Herbert G Brick et W Saunders il semble qu une *pathogenie* identique soit probable pour tous les cholesteatomes acquis[1]

1 Chaque cholesteatome se compose d une matrice prenant naissance des cellules basales de l epithelium du tympan ou de la peau du conduit auditif externe voisine du tympan et d un substrat mesenchymal la perimatrice (D I Lim et collaborateurs) qui active et dirige la croissance

2 Cette croissance s etablit apres la destruction ou le ramollissement de la membrane basale

3 Des phenomenes inflammatoires dans l oreille moyenne et exceptionnellement dans le conduit auditif externe stimulent les cellules basales et possedant un potentiel de croissance penetrent avec les proliferations solides (matrice) à travers la membrane basale defectueuse dans un substrat mésenchymal (perimatrice)

4 La perimatrice se compose d un tissu conjonctif subepithelial inflammatoire ou d un tissu granulomateux nouvellement forme Dans le cholesteatome de l attique la perimatrice consiste en plusieurs couches d un tissu conjonctif lache a la suite d une pneumatisation incomplete

5 La croissance active de la matrice s arrete au niveau de l os Un tissue granulomateux couvre l os nu Bruce J Gantz et ses collaborateus ainsi que Maxwell Abramson et ses collaborateurs trouverent dans le tissu granulomateux des enzymes resorbant l os

6 Dès ce moment commence la 2me phase plutot passive de la croissance du cholesteatome L exfoliation de lamelles epitheliales continue et le sac cholesteatomateux s elargit Alors que M Abramson rejette une resorption osseuse provoquee par la pression du materiel cholestéatomateux je suis encore toujours convaincu que les changements de pression continuels à l interieur des masses cholestéatomateuses stimulent l activite resorbante du tissu granulomateux

7 Durant les 2 phases de la croissance du cholestéatome quelques proliférations penetrent dans l oreille moyenne et percent la membrane de Shrapnell d ou resulte une perforation de l attique Derriere cette perforation on decouvre la presence d un grand cholestéatome

8 La therapie de chaque cholesteatome consiste a eloigner toute la matrice et egalement toute la perimatrice

Du moment ou les proliferations cholestéatomateuses atteignent la paroi posterieure du tympan seule la creation d une cavite ouverte permet le nettoyage necessaire de l oreille moyenne et du systeme cellulaire communiquant Les recidives frequents après la tympanotomie posterieure superieure (canal up) (Cl Jansen) sont causes par l eloignement incomplet de la matrice et surtout de la perimatrice plutot que par une dysfonction de la trompe d Eustache

Si l on soupçonne un cholesteatome derriere des irregularites de la membrane de Shrapnell une operation preventive est indiqueee

ZUSAMMENFASSUNG

Nach der Bestatigung von Manasses und Langes Befunden durch O Steurer, W Albrecht und M Schwarz und unseren Feststellungen von Irrtumern in Wittmaacks Doktrin, ist die Retraktionstheorie von Bezold durch die meisten der deutschlesenden Otologen aufgegeben worden

Die Auswirkungen der Infektionen auf die *normale Entwicklung der Mittelohrraume* wurde untersucht Aufgrund dieser Resultate, und kombiniert mit neuen Beobachtungen von David J. Lim, Herbert G Brick und W Saunders, erscheint eine identische Pathogenese aller erworbenen Cholesteatome wahrscheinlich

1. Jedes Cholesteatom setzt sich zusammen aus einer Matrix, entstehend aus den Basalzellen des Epithels des Trommelfells oder der ans Trommelfell anstossenden Gehorgangshaut und aus einem mesenchymalen Substrat, der Perimatrix (D J Lim und Mitarbeiter), welches das Wachstum der Matrix fördert und leitet

2 Dieses Wachstum findet statt nach der erstorung oder der Auflockerung der Basalmembran

3 Entzundliche Vorgange im Mittelohr und ausnahmsweise auch im Gehorgang regen die potenten Basalzellen an, mit soliden Zapfen (Matrix) durch die defekte Basalmembran in ein mesenchymales Substrat (Perimatrix) hineinzuwachsen

4 Die Perimatrix besteht aus entzundetem subepithelialem Bindegewebe oder aus neugebildetem Granulationsgewebe Im Attik Cholesteatom besteht die Perimatrix aus submukosen Lagen von lockerem Bindegewebe infolge einer unvollstandigen Pneumatisation

5 Das aktive Wachstum der Matrix stoppt am Knochen Granulationsgewebe bedeckt den nackten Knochen Bruce J Gantz und seine Mitarbeiter und Maxwell Abramson und seine Mitarbeiter fanden im Granulationsgewebe knochenresorbierende Enzyme

6 Damit beginnt eine zweite, mehr passive Phase des Cholesteatom Wachstum Die Abschilferung von epithelialen Lamellen setzt sich fort, und der Cholesteatomsack weitet sich aus Wahrend M Abramson einen Druck *des cholestatomatösen Materials, welches an* der Knochenresorption beteiligt ist, ablehnt, bin ich immer noch uberzeugt, dass fortgesetzte Druckschwankungen innerhalb der *Cholesteatom-Massen die Knochenresorbierende* Aktivitat des Granulationsgewebes anregen

7 Wahrend den zwei Phasen des Cholesteatom-Wachstums brechen einige Cholesteatom-Schlauche ins Mittelohr hinein und durch die Shrapnell sche Membran hindurch, woraus eine Attik-Perforation entsteht Hinter dieser Perforation wird ein grosses Cholesteatom entdeckt

Die *Behandlung* aller Cholesteatome muss die ganze Matrix, und ebenfalls die ganze Perimatrix entfernen

Sobald die Cholesteatom-Schlauche die Hinterwand des Trommelfells erreicht haben, ermoglicht nur die Schaffung einer offenen Hohle die notwendige Reinigung des Mittelohres und der kommunizierenden Zell Systeme Die haufigen Ruckfälle einer Cholesteatom Bildung nach der posterior superioren Tympanotomie (canal up) Technik (Cl Jansen) werden verursacht durch die unvollstandige Entfernung der Matrix und besonders der Perimatrix, viel eher als durch eine Dysfunktion der Eustachischen Rohre

Durch eine praventive Operation von verdächtigen Fällen sollte die Zahl der Shrapnell (attic) Cholesteatome vermindert werden

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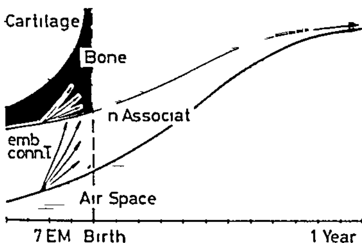


Fig. 1

2:1 2:2

2:3 2:4

Fig. 2. Vertical sections of the antrum region in four newborns. Numbers refer to cases mentioned in text (compare No. 2:3 with Fig. 3).

Fig. 3 Higher magnification of antrum No. 2 3 of Fig 2.

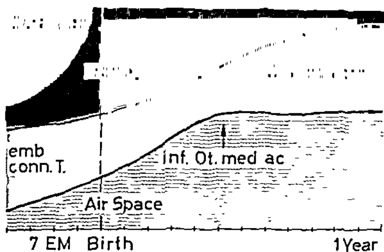


Fig 4



Fig 5 A 6-month-old baby boy Vertical section through tympanic cavity Acute otitis media Submucous connective tissue on the floor on the promotory and within the epitympanic space



Fig 6 Same case as in Fig 5 Vertical section through mastoid process Infantile acute otitis media Dissociation of the two cavity building processes Pneumatization inhibited Bone cavity performation still going on

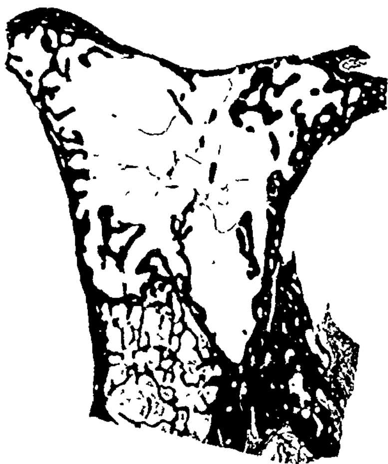


Fig 7 A 4½ year old girl Vertical section through mastoid process Adhesions and dissociation Bone cavity formation is quite extensive, whereas pneumatization peripherally incomplete Large amount of submucous connective tissue



Fig 8 A 4½ year old girl Vertical section through tympanon Adhesive processes and dissociation within the epitympanic space



Fig 9 A 4½ year-old girl Picture in detail of Fig 8 Behind cholesteatoma membrane (*S*) the epitympanic space is filled by loose connective tissue (*B*) *H* Head of the malleus

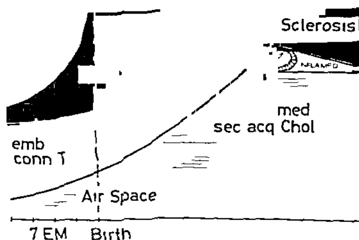


Fig 10





Fig 11 Necrotizing otitis media A cone of basal cells (Matrix *M*) invades the submucous tissue (Perimatrix *P*)

Fig 12 Secondary acquired cholesteatoma in an 11 year old boy Matrix growing within submucous perimatrix

Fig 13 Same case as in Fig 12 The submucous growing matrix breaks through the inflamed mucosa into a mastoid cell

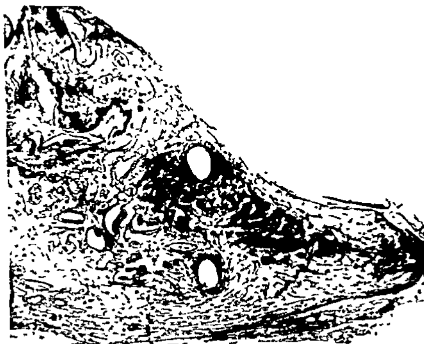


Fig 14 A 19½ year-old woman Horizontal section through the temporal bone Cholesteatomatous columns penetrate the submucous tissue

M

P

15



Fig. 15. Same case as in Fig. 14. New bone formation in the neighborhood of growing basal cone of cholesteatoma (matrix *M*) (perimatrix *P*).

Fig. 16. A 30-year-old woman. Horizontal section across the attic flattened matrix covering the bone (granulation tissue *G*).



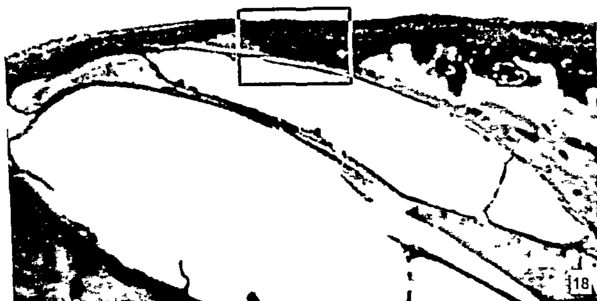


Fig 17 Normal mucoperiosteum

Fig 18 Same case as in Fig 16 Cholesteatomatous matrix squeezed against bony wall of the basal cochlear turn Fistula formation (within box)



Fig. 19. Higher magnification of Fig. 18 (box). A large granulation tissue with many capillaries below the matrix.

Fig. 20. Healthy newborn with typical hyperkeratosis and acanthoses in the fundus of the external meatal canal.



Fig 21 Vertical section through the external auditory meatus and Shrapnell's membrane in the case of an 11 month-old baby girl suffering from acute otitis media

Fig 22 Higher magnification of Fig 14 Cone of basal cells (matrix *M* growing into perimatrix *P*)



24

Fig 23 A 68 year old man Chronic eczema of external meatus Vertical section through Shrapnell's membrane Ingrowing basal cells (matrix *M*) into inflamed connective tissue (perimatrix *P*)

Fig 24 A 23 year old man Vertical section of a Shrapnell s cholesteatoma in the right ear

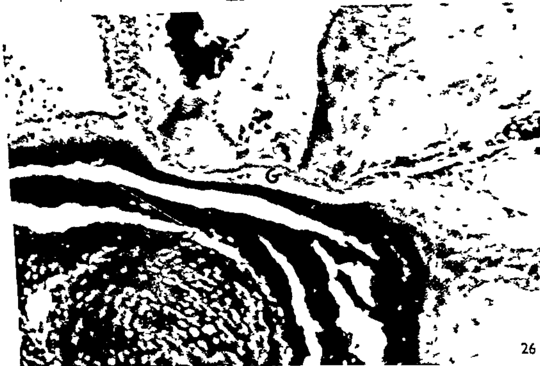


Fig. 25. Same case as in Fig. 24. The active growth of matrix (*M*) proceeds alongside the osteogenetic process taking place in the same connective tissue (perimatrix).

Fig. 26. Same case as in Fig. 24. Transition from the primary active phase of cholesteatoma growth to the secondary passive phase where the matrix contacts the bone (granulation tissue *G*).

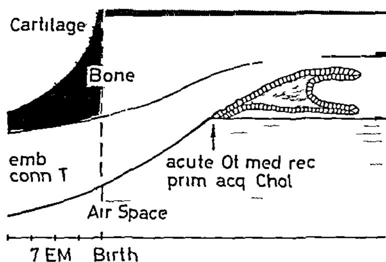


Fig 27

Fig 28 A 23 year-old man Cholesteatomatous matrix (*M*) growing within connective tissue (perimatrix *P*)



Fig. 29. Experimental cholesteatoma in a guinea pig growing out of intact tympanic membrane as matrix (*M*) into granulation tissue (perimatrix *P*).

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SUPPLEMENT 362

INDIRECT DETERMINATION OF VARIATIONS IN THE INNER EAR PRESSURE IN MAN

An experimental study

by

Margaretha Casselbrant

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INDIRECT DETERMINATION OF VARIATIONS IN THE INNER EAR PRESSURE IN MAN

An experimental study

by

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Malmo 1979

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The present thesis is based on the following papers which will be referred to in the text by Roman numerals:

I. Casselbrant, M., Ingelstedt, S. & Ivarsson, A.

Volume displacement of the tympanic membrane in the sitting position as a function of middle ear muscle activity. A quantitative microflow method.
Acta Otolaryngol (Stockh) 84: 402-403, 1977

II. Casselbrant, M., Ingelstedt, S. & Ivarsson, A.

Volume displacement of the tympanic membrane at stapedius reflex activity in different postures. Studies on variations in the perilymphatic pressure.
Acta Otolaryngol (Stockh) 85. 1-9, 1978

III. Casselbrant, M.

Experimental studies on the stapedius reflex response and the influence of different perilymphatic pressures in human temporal bones.
Page 31-42.

IV. Casselbrant, M. & Henriksson, B.

Experimental studies on the bio-mechanics of the incudostapedial joint in human temporal bones.
Page 43-50.

Introduction

Considerable attention has for many years been paid to the hydrostatic pressure of the inner ear fluids and to the problem as to whether or not the cerebrospinal fluid pressure changes are reflected in the perilymph For obvious reasons direct recordings of the inner ear pressure in man cannot be made, so investigators have had to use indirect recording methods Two conditions must be fulfilled in successful studies of inner ear pressure variations in man First, to record such variations from outside the inner ear, and secondly, to induce such pressure changes for experimental studies This involves certain practical problems In previous studies these problems have been tackled in different ways, by using human temporal bones and by using animals for indirect as well as direct recordings of pressure variations in the perilymph

Thus, Bekesy (1942), Metz (1946) and Andersen et al (1962) tried to find out if an increased inner ear pressure could be shown to have any effect on the sound transmission in human temporal bones Metz (1946) was able to record changes in the acoustic impedance when the inner ear pressure in creased He used an impedance bridge and made the recordings from outside the eardrum Bekesy (1942) and Andersen et al (1962), on the other hand, could not find that an incrased inner ear pressure exerted any influence on sound transmission They studied the sound transmission between the tympanic membrane and the round window

In experiments on animals it has been shown, by direct recordings, that changes in the cerebrospinal fluid pressure are reflected in the perilymphatic pressure and that this pressure transmission takes place mainly via the cochlear aqueduct (Ahlen, 1947, Kerth & Allen, 1963, Bentjees, 1972, Carlborg et al , 1979)

By means of cochlear microphonics it has also been shown in animals that a reduction in response occurs when the inner ear pressure is increased by applying the pressure to the labyrinth (McCabe & Wolsk, 1961, Butler & Honrubia, 1963, Allen et al , 1971) or by raising the cerebrospinal fluid pressure (Allen & Habibi, 1962, Feldman, 1968, Benitez, 1972) Klockhoff et al (1965) could record a change in the acoustic impedance when the cerebrospinal fluid pressure was increased During the experiment the bulla was opened to eliminate any possible changes in the middle ear

pressure

Opinions about the patency of the human cochlear aqueduct, however, differ widely (Karlefors, 1924, Perlman & Lindsay, 1939, Waltner, 1948, Allen, 1964, Anson et al , 1964, 1965, Holden & Schuknecht, 1968, Palva & Dammert, 1969, Rask-Andersson et al , 1977 and Wlodyka, 1978) It should be observed, however, that most of these studies are histological investigations A clinical indication of a patent cochlear aqueduct is fluid welling up from the vestibulum during stapes surgery (Shea, 1963, Schuknecht, 1971 and Van Doren Hough, 1976). Other pathways along which intracranial pressure can be transmitted to the inner ear are the accessory canals of the cochlear aqueduct, the endolymphatic duct, the perineural space and the vascular bed of the inner ear

The intracranial pressure in man is known to increase by about 1 kPa (10 cm H_2O) when the posture is changed from sitting to recumbency (Best & Taylor, 1945) This pressure increase is mainly an effect of a hydrostatic venous pressure increase (Dawson, 1967). Recordings of the pressure in the internal jugular vein, at the level of the skull base, have shown an increase by about 1 kPa induced by change in posture from sitting to recumbency (Ingelstedt et al , 1967, Jonson & Rundcrantz, 1969) Furthermore, Tjernstrom (1974) showed that for eliciting Alternobaric Vertigo in the recumbent position the middle ear pressure had to be increased by a further 1 kPa as compared with the sitting position It thus seems possible in experimental studies in man to use the hydrostatic venous pressure increase induced by change in posture or by compression of the neck veins in order to find out if pressure variations in the inner ear could be recorded from outside the tympanic membrane

In recent studies of normal subjects, indirect methods such as recordings of hearing thresholds, acoustic impedance and stapedius reflex response have been used with the intention to find out if it is possible to record changes in the inner ear pressure as an effect of change in posture or of compression of neck veins Thus Miltich (1968) and Macrae (1972) found a threshold shift with change in posture from sitting to inverted position Macrae (1972, 1974) could also demonstrate changes in the acoustic impedance Changes in the stapedius reflex response were demonstrated by Klockhoff et al (1965) using acoustic impedance as an effect of compression of neck veins and by Brask (1978) using extratympanic manometry as an effect of change in posture All these authors considered their recorded

response change to be an effect of a change in the inner ear pressure. Macrae (1974), however, was the only one who considered a change in the middle ear pressure, induced by change in posture, to be a source of error.

Change in posture as well as compression of the neck veins will cause an increase of blood in the vessels of the middle ear mucosa but also in the vessels of the skin of the external ear canal, i.e. a congestion due to increase in hydrostatic pressure. This induces an increase in the middle ear pressure due to the reduction in volume of the airfilled middle ear space and also a reduction in volume of the external ear canal. Studies of pressure variations in the inner ear in man, induced by change in posture, thus require a completely equilibrated middle ear pressure, i.e. the subject has to be able to equilibrate completely the middle ear in both sitting and recumbent positions. Furthermore, it is obvious that 'closed methods", such as acoustic impedance and extratympanic manometry, cannot be used since the resulting change in volume of the external ear canal may prove to be an important source of error.

Ivarsson (1975) has shown that about 70 % of the tympanic membrane displacement recorded with a change in posture from sitting to recumbency is due to the change in volume of that part of the external ear canal which is 'closed in" between the tympanic membrane and the earcuff, and that only about 30 % of the displacement is due to changes in the perilymphatic pressure. These recordings were made on a subject who could keep the Eustachian tube open voluntarily in all positions, i.e. the middle ear pressure was completely equilibrated. In experiments on the same subject, only 10 % of the recorded tympanic membrane displacement was induced by change in the perilymphatic pressure while the Eustachian tube was kept closed during change of posture. This indicates that change in posture alone cannot be used for recording variations in the perilymphatic pressure with closed methods of investigation, even if the middle ear pressure is equilibrated

Thus the prerequisites for recording pressure variations, induced by change of posture in the inner ear from the outside of the tympanic membrane, are (1) a completely equilibrated middle ear pressure, i.e. there must be no pressure gradient across the tympanic membrane; and (2) a method by which the error induced by a change in the external ear canal volume could be eliminated

Such a method was introduced by Elner, Ingelstedt & Ivarsson (1971) with the integrating microflow method, which involves a free communication between the tympanic membrane and the ambient pressure.

The main purpose of the present studies was to find out if it is possible to demonstrate changes in the perilymphatic pressure with a change in posture by quantitative recordings of the tympanic membrane displacement caused by the stapedius muscle contraction. The investigation was made on normal subjects and attempts were made to reproduce and explain by experiments on human temporal bones.

I. VOLUME DISPLACEMENT OF THE TYMPANIC MEMBRANE IN THE SITTING POSITION AS A FUNCTION OF MIDDLE EAR MUSCLE ACTIVITY

A quantitative microflow method

For clinical measurements, the stapedius reflex responses have generally been recorded qualitatively using acoustic impedance bridges.
The integrating microflow method devised by Elner et al. (1971) (see Fig. 1). has made it possible to record the direction of movement as well as the quantitative displacement of the tympanic membrane caused by a stapedius muscle contraction.

The aim of the present paper is to investigate the acoustically elicited stapedius reflex response with this quantitative method and with regard to the following factors.

1. Stimulus frequency
2. Stimulus intensity
3. Stimulus duration
4. Influence of the middle ear pressure

Subjects with normal ears have been investigated in the sitting position. These subjects could equilibrate the middle ear pressure completely, i.e. they belonged to tubal function group Ib ad modum Elner et al. (1971).

As the stapedius reflex was elicited in the contralateral ear, the different factors have been discussed in regard to Tone and Probe ear.

PRESSURE CHAMBER

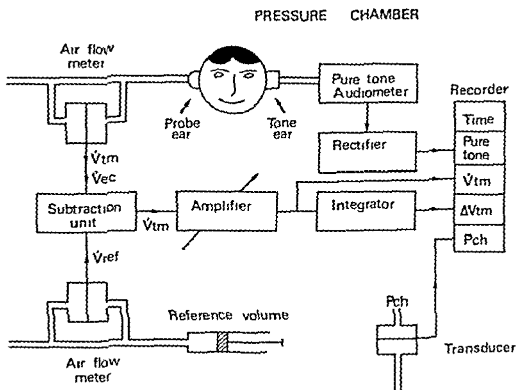


Figure 1. Schematic diagram of the equipment. Symbols: $\dot{V}_{tm}$, Air flow velocity through the resistor of the ear canal flowmeter caused by the volume deviation of the tympanic membrane. $\dot{V}_{ec}$, Air flow velocity through the resistor of the ear canal flowmeter caused by compression or expansion of the gas in the external ear canal and in the flowmeter system by change in the ambient pressure. $\dot{V}_{ref}$, Flow velocity through the resistor of the reference flowmeter caused by compression or expansion of gas in the reference system by change in the ambient pressure. P_{ch}, Pressure in the pressure chamber, i e. the ambient pressure V_{tm}, Volume deviation of the tympanic membrane in relation to its neutral position. Δ before the symbol indicates a change of the variable.

STIMULUS CHARACTERISTICS IN TONE EAR

Stimulus frequency

The stapedius reflex response was investigated at the sound frequencies 0.5, 1, 2 and 4 kHz. The lowest reflex threshold was recorded at 1 kHz (Median 90 dB, Quartiles 85, 95 dB).

Stimulus intensity

The sound stimulus was given in the contralateral ear at different intensities up to 105 dB rel ISO 1964. The intensity was increased in stage of 5 dB.

The reflex response amplitude (Probe ear) was found not to increase above a stimulus intensity of 95 dB for 75 % of the ears In the remaining ears there was only a minor increase in the amplitude up to a stimulus intensity of 105 dB.

Latency of reflex response (Probe ear) was determined at the stapedius reflex threshold (50-230 ms) and at a stimulus intensity of 5 dB above the threshold (30-140 ms). As the stimulus intensity increase was 0.3 dB/ms it was not possible to estimate the latency for higher stimulus intensity than 5 dB above the threshold.

At the sound stimulus 105 dB, 1 kHz, a superimposed reflex response was occasionally recorded in 3 subjects which showed a longer latency (mean 650 ms) as compared with the latency of the stapedius reflex (mean 107 ms at threshold level).

Rise time of reflex response (Probe ear) was determined at the stapedius reflex threshold (80-330 ms) in 5 dB step up to 20 dB above the threshold (40-90 ms)

Stimulus duration

Initial and permanent phase of the reflex response amplitude (Probe ear) was recorded and the initial response amplitude was greater than the continuous permanent outward or inward movement of the tympanic membrane. The reproducibility of the two phases were high considering pulse and

breathing which might disturb the permanent phase (see Fig 2)

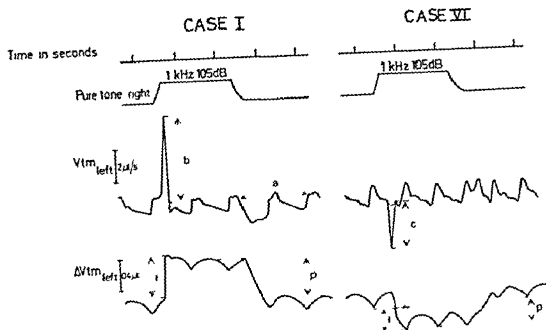


Figure 2 Recording in two cases V_{tm} represents the recorded flow velocity at an outward (b) or inward (c) movement of the tympanic membrane and movements of the tympanic membrane synchronous with the pulse (a) ΔV_{tm} represents the integrated flow velocity corresponding to the volume deviation of the tympanic membrane i = initial, p = permanent phase of the volume displacement of the tympanic membrane at stapedius muscle contraction

)

Temporary threshold shift (TTS) ("one ear) had to be taken into consideration, as the acoustic stimulus 105 dB, 1 kHz, was repeated more than 100 times in each ear during the investigations (3 hours) A TTS might affect the reflex response amplitude, so the stimulus duration must be as short as possible The shortest stimulus duration required to elicit a maximum reflex response for a pure tone 1 kHz, at threshold level, is 130-560 ms The reproducibility of the quantitative stapedius reflex response was high, which indicates that a TTS was negligible in this investigation at an acoustic stimulus of 1 kHz, 105 dB and a stimulus duration of about 2 seconds

MIDDLE EAR PRESSURE IN TONE EAR

The absolute hearing threshold at 1 kHz was determined with Békésy audiometry at different over- and underpressures in the middle ear in order to evaluate its influence on the sound transmission. The hearing threshold was changed Median 6.5 dB (Quartiles 4, 9 dB) when the middle ear pressure was ±1.5 kPa (±15 cm H_2O). Thus even if the middle ear pressure in the Tone ear is ±1.5 kPa the reflex response amplitude is not affected since the stimulus intensity is 105 dB, as it was shown that the reflex response amplitude reached a plateau at a stimulus intensity of 95 dB in most of the ears

MIDDLE EAR PRESSURE IN PROBE EAR

The influence on the reflex response amplitude (105 dB, 1 kHz) of different middle ear pressures in the Probe ear was studied with the aid of a pressure chamber Relative over- and underpressures (±0.2, 0.4, 0.6, 1.0 and 1 5 kPa) were created in the middle ear when the subject was instructed not to swallow. The reflex response amplitude was reduced when the tympanic membrane was outside or inside its neutral position. In some ears there was a change from an outward to an inward movement of the tympanic membrane, and the reflex response could even disappear.

CONCLUSIONS

As shown by Mendelsson (1966) and Lidén et al (1970), an acoustically elicited stapedius muscle contraction causes either an outward or an inward movement of the tympanic membrane.
When measuring the threshold of the stapedius reflex using the integrating microflow method devised by Elner et al. (1971), the sensitivity of this method was found to be about the same as that of the impedance method in the frequency range 250-2000 Hz (Jerger et al. 1972).
The latency of the stapedius reflex was shown to decrease with increasing stimulus intensity, which is in accordance with the results presented by Metz (1951) and Møller (1958).

The acoustic stimulus, 105 dB, 1 kHz, was selected as test stimulus, since it provided the greatest reflex response amplitude. Using this stimulus intensity a superimposed reflex response was recorded occasionally. This reflex response had a longer latency and was always recorded as a momentarily vigorous inward movement of the tympanic membrane, which could easily be separated from a stapedius reflex. This reflex response was assumed to be a // tensor tympanic contraction.

A pressure gradient of ±1.5 kPa (±15 cm H_2O) across the tympanic membrane in the Tone ear reduced the sound transmission, but not to such an extent that the reflex response amplitude was changed. On the other hand, it was found that even such a small pressure gradient across the tympanic membrane as 0 1 kPa (1 cm H_2O) in the Probe ear could markedly affect the reflex response amplitude. The amplitude was usually greatest when the middle ear pressure was equal to ambient pressure, which clearly demonstrates the importance of testing the middle ear pressure in the Probe ear before clinical measurements of the stapedius reflex response are made.

II. VOLUME DISPLACEMENT OF THE TYMPANIC MEMBRANE AT STAPEDIUS REFLEX ACTIVITY IN DIFFERENT POSTURES

Studies on variations in the perilymphatic pressure

It might be assumed that the perilymphatic pressure changes with posture in the same way as the cerebrospinal pressure, which has been shown to increase by about 0.1 kPa when the posture is changed from the erect to the horisontal position (*Best & Taylor, 1945*).
This pressure increase has been assumed to be mainly an effect of a hydrostatic venous pressure increase (Dawson, 1967). In a study by Jonson & Rundcrantz (1969) it was shown that the pressure in the internal jugular vein, at the level of the skull base, increases by about the same value with a change in posture. It would therefore seem possible to use a change in posture for studies of changes in the perilymphatic pressure.

In paper I a method was presented which allows quantitative recordings of the tympanic membrane displacement caused by a stapedius muscle contraction. Since it might be assumed that variations in the perilymphatic pressure would cause variations in the quantitative stapedius reflex response due to different pressures loading the stapedial footplate, it was considered important to find out if such changes in the stapedius reflex response could be recorded indirectly by means of the method described in paper I.

The main purpose of the present study was to find out if it is possible to record any changes in the stapedius reflex response as induced by changes in posture.

Paper I showed the importance of testing the middle ear pressure in the Probe ear before recordings were made of the stapedius reflex response, as even such a small pressure gradient across the tympanic membrane as 0 1 kPa could markedly affect the quantitative response. The middle ear pressure was therefore tested in the sitting as well as in the recumbent position. An average increase in the middle ear pressure of 0,1 kPa was found when the subject assumed a recumbent position and before the middle ear pressure was equilibrated. This pressure increase could be explained by the congestion of the middle ear mucosa, reducing the airfilled middle ear space as an effect of the recumbent position (Andréasson et al., 1976). This source of error, which mars the recording of the quantitative stapedius reflex response, forced us to select subjects for the present study who could equilibrate their middle ears perfectly in the sitting as well as

in the recumbent position

The stapedius reflex response in sitting and recumbency

The tympanic membrane displacement caused by an acoustically elicited stapedius reflex response (1 kHz, 105 dB) was recorded quantitatively both in the sitting and the recumbent position and the recordings were always done after equilibration of the middle ear pressure A change in the reflex response was seen with change in posture and three patterns were observed

1) a pronounced outward displacement of the tympanic membrane in the sitting position was less pronounced in recumbency (Fig 1),
2) a small outward displacement of the tympanic membrane in the sitting position changed to an inward displacement in recumbency,
3) an inward displacement of the tympanic membrane in the sitting position was more pronounced in recumbency (Fig 1)

The examinations were repeated at intervals of several months in three subjects and the results showed a high reproducibility between the different examinations

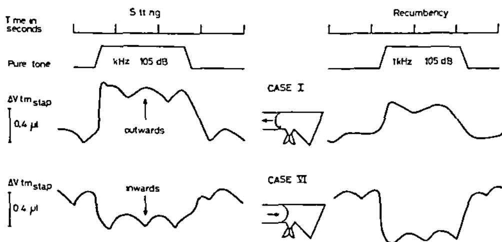


Figure 1 Recording in two cases of outward (case 1) or inward (case VI) movements of the tympanic membrane at stapedius muscle contraction in sitting and recumbent positions

Gravitational force exerted on the ossicular chain at a change in posture

In order to find out if the gravitational force might have any appreciable influence on the reflex response, the following experiments were performed

The stapedius reflex response, measured as the tympanic membrane displacement, was recorded in different postures, gradually changing from sitting (85°) to recumbency (0°) The essential change in the reflex response occured in the positions between 0-10° above the horizontal plane This change in response might reflect a change in perilymphatic pressure according to the results of Jonson & Rundcrantz (1969) who found that the greatest change in venous pressure occurred within the same body positions

Further examinations were also performed in order to preclude the possibility that gravitational forces might be responsible for the differences in reflex response which were observed when the results obtained in the sitting and the recumbent positions were compared

The stapedius reflex response was recorded on a sitting subject and during a period of compression of the neck veins with a pressure of 4 kPa (30 mm Hg) This neck vein compression is known to correspond to the increase in venous pressure induced by a change in posture from sitting to recumbency (Jonson & Rundcrantz, 1969) The tympanic membrane displacement caused by a stapedius reflex during this experimental condition was of the same magnitude as that recorded in recumbency The middle ear pressure was equilibrated to atmospheric pressure during both examinations

CONCLUSIONS

A comparison of the results obtained on subjects in the sitting and the recumbent positions and with equilibrated middle ear pressure shows a regular change in the stapedius reflex response, recorded as the quantitative displacement of the tympanic membrane In some ears there was also a change in the direction of the tympanic membrane displacement We assume that the recorded differences are caused by an increase in the perilymphatic pressure as an effect of a change in posture

This assumption is supported by Densert et al (1977), who in investigations of human temporal bones found that changes in the perilymphatic pressure could affect the displacement of the tympanic membrane caused by a simula-

ted stapedius muscle contraction.

The investigations of the reflex response at gradual changes in posture as well as during neck vein compression showed that the differences in the reflex response could not be caused by a changed gravitational force exerted on the ossicular chain.

The results indicate that the perilymphatic pressure in man varies with posture and that these variations can be recorded indirectly on the outside of the tympanic membrane. A prerequisite is, however, that the subjects are able to equilibrate their middle ears both in the sitting and recumbent postures, since even small pressure gradients across the eardrum could affect the reflex response.

A hypothesis was presented to explain the outward or inward displacement of the tympanic membrane caused by a stapedius muscle contraction. This displacement could probably be explained by differences in the anatomical relation between the pyramidal eminentia, the stapes, and the stapedial tendon.

III. EXPERIMENTAL STUDIES ON THE STAPEDIUS REFLEX RESPONSE AND THE INFLUENCE OF DIFFERENT PERILYMPHATIC PRESSURES IN HUMAN TEMPORAL BONES

The acoustically elicited stapedius muscle contraction is known to cause either an outward or an inward movement of the tympanic membrane (Mendelson, 1966, Lidén et al., 1970, Casselbrant et al., 1977; Brask, 1978). Casselbrant et al. (1978) however, could also demonstrate that change in posture from sitting to recumbency affects the reflex response quantitatively, and three different patterns of response were seen (paper II).

The aim of this study was to found out (a) if it is possible to reproduce all three patterns of reflex response in human temporal bones by changing the perilymphatic pressure and (b) if it is possible to find a mechanical explanation of the different directions of movement of the tympanic membrane.

PERFORMANCE AND RESULTS

Stapedius muscle contractions were simulated by loading the stapedius muscle intermittently with weights of 5, 10 or 15 g, and the volume displacement of the tympanic membrane caused by these loadings were recorded by means of the integrating microflow method devised by Elner et al. (1971) Series of such recordings were done with different overpressures (0.5, 1, 1 5 kPa) applied to the perilymphatic space via a fistula into the superior semicircular canal to which a pressure unit was connected

These recordings showed that the displacement of the tympanic membrane increased with heavier weights loading the stapedius muscle. Furthermore, quantitative changes as well as changes in direction of movement of the tympanic membrane were seen when recordings were made with different degrees of overpressure applied to the perilymphatic space. Thus, when summing up the results from these experiments it was demonstrated that all three patterns of reflex response seen to occur in man with change in posture, could be reproduced and explained by changes in the perilymphatic pressure

In another series of experiments in which the flowmeter in use was connected to the fistula of the semicircular canal, it was possible to record the

displacement of the perilymph induced by movements of the stapedial footplate For these experiments were used temporal bones with an inward as well as an outward movement of the tympanic membrane as an effect of a simulated stapedius muscle contraction The results of these experiments showed that the direction of perilymph displacement caused by the stapedial footplate movement induced by a simulated stapedius muscle contraction was always the same This direction could only be induced by an increase in the perilymphatic space, i e the stapedial footplate always moved laterally towards the middle ear These findings were seen independently of the temporal bone used, i e the same pattern was seen whether the tympanic membrane was displaced inwards or outwards as the effect of the stapedius muscle contraction

The final experiments were performed in order to find out if the direction of the tympanic membrane displacement was the same as the direction of movement of the long process of the incus These experiments were performed by a careful manipulation of the long process of the incus using an operating needle, and the results showed that the tympanic membrane always moved in the same direction as the long process of the incus, and furthermore, these results were seen independently of the temporal bone used for the experiments, i e whether a simulated stapedius muscle contraction caused an inward or an outward movement of the tympanic membrane During all experiments mentioned above the middle ear pressure was always in open communication with the atmosphere

CLUSIONS

results of the present study have thus shown that the quantitative nges of the stapedius reflex response, recorded as the displacement of tympanic membrane and induced by change in posture, could be explained by changes in the perilymphatic pressure as an effect of the different body positions

It might also be concluded that it is possible to record variations in the perilymphatic pressure from the outside of the tympanic membrane, provided that the middle ear pressure is completely equilibrated

It was also found that the mechanical explanation of the outward or inward movement of the tympanic membrane must be looked for in the incudostapedial

joint since the stapedial footplate always moved laterally, i.e. towards the middle ear. Also the direction of the tympanic membrane displacement was always the same as the movement direction of the long process of the incus.

IV. EXPERIMENTAL STUDIES ON THE BIO-MECHANICS OF THE INCUDO-STAPEDIAL JOINT IN HUMAN TEMPORAL BONES

Experimental studies on human temporal bones have shown that independently of the movement of the tympanic membrane, the movement of the stapedial footplate caused by a simulated stapedius muscle contraction always induces an increase in the perilymphatic space Nor is there any difference in the movement direction of the tympanic membrane and the crus longum incudis, when this is manipulated towards or away from the tympanic membrane (Paper III)

The aim of present investigation has been to try and find the explanation of the outward or inward movement of the tympanic membrane caused by a simulated stapedius muscle contraction, by analysing the movements of the incudostapedial joint

PERFORMANCE AND RESULTS

Via an opening in the jugular fossa, photos were taken of the incudo-stapedial joint at a stapedius muscle contraction simulated by loading the muscle with weights A double exposure photo technique was used, i e one exposure was made when the muscle was unloaded and the other when it was loaded Whether there was an outward or an inward movement of the tympanic membrane, the loading of the stapedius muscle caused a sliding ovement in the incudostapedial joint The stapes made a rotary movement round an axis close to the posterior end of the stapedial footplate, and e head of the stapes was moved towards the pyramidal eminentia At the ame time the incus made a sliding movement and in some temporal bones, e processus lenticularis was seen to move a small distance in the same direction, but the movement of the stapes was always greater Apart from the sliding movement in the incudostapedial joint, a translatory movement of the processus lenticularis towards or away from the tympanic membrane was also recorded

Thus the differences in the movement of the stapes and the processus lenticularis recorded at a simulated stapedius muscle contraction were probably due to the biomechanics of the incudostapedial joint
Using a scanning electron microscope the articular surface of the head of

the stapes were investigated separately after the incudostapedial joint had been opened and the stapes and the incus had been extracted and all soft tissue removed. The anatomical investigations showed that the articular surface of the head of the stapes were highly variable in shape from one specimen to another, but from a functional point of view two different types could be identified.

Thus as a result of the shapes of the articular surfaces, contraction of the stapedius muscle causes a sliding movement in the incudostapedial joint and in temporal bones with an outward movement of the tympanic membrane, the processus lenticularis slides upwards since the anterior margin of the articular surface was always higher than the central part. On the other hand, in temporal bones with an inward movement of the tympanic membrane, the processus lenticularis will slide downwards since the anterior margin is lower than the central part. Consequently, when the incus is moved towards respectively away from the tympanic membrane, this causes the tympanic membrane to move outwards or inwards, which has been shown in Paper III.

The reults of the present investigations show that it is possible to explain the outward or inward movement of the tympanic membrane caused by a simulated stapedius muscle contraction by differences in the articular surface of the head of the stapes.

General discussion

A great deal of attention has been paid to the direction of the tympanic membrane displacement caused by the stapedius muscle reflex. For many years it was considered that a contraction of the stapedius muscle caused an outward movement of the tympanic membrane, while a contraction of the tensor tympani muscle caused an inward displacement of the tympanic membrane (Terkildsen, 1957, Mendelson, 1961; Weiss et al.,1962; Holst et al., 1963.

In 1966 Mendelsson and in 1970 Lidén et al. could demonstrate that a contraction of the stapedius muscle could cause an outward as well as an inward displacement of the tympanic membrane. Their findings were confirmed in 1977 by Casselbrant et al. and in 1978 by Brask.

The only method so far presented which allows a quantitative measure of the displacement of the tympanic membrane is the open microflow method devised by Ingelstedt et al. (1967) and further developed by Elner et al. (1971). By means of this method it was possible, in the present work, to demonstrate quantitative changes of the stapedius reflex response induced by change in posture. It was also possible in some ears to find a change in the direction of the displacement with a change in posture.

This change in the reflex response was assumed to be induced by a change in the perilymphatic pressure as an effect of a change in posture, and the assumption was tested by experiments in human temporal bones. It was then shown that all changes in the reflex response which are seen to occur in man with a change in posture, could be explained by changes in the perilymphatic pressure Thus, the investigations presented in this thesis show that it is possible to record variations in the perilymphatic pressure indirectly, from the outside of the tympanic membrane.

Certain reservations, however, must be discussed. It has been known for a long time that a pressure gradient of a certain degree across the eardrum might conceal the stapedius reflex response as recorded from the tympanic membrane displacement (Terkildsen, 1960, Klockhoff, 1961, Djupesland, 1969, Petersen & Lidén 1972, Renvall & Holmqvist, 1976) In the present study it was shown that such a small pressure gradient as 0 1 kPa across the tympanic membrane considerably influenced the response in some ears, and it was also seen that a change from sitting to recumbent posture caused a change in the middle ear pressure of about 0 1 kPa Thus, subjects used

for recordings of the perilymphatic pressure variations ought to be able to equilibrate the middle ear pressure completely, also in recumbency.

In human temporal bones the tympanic membrane displacement could be recorded simultaneously with a change in the perilymphatic pressure while maintaining atmospheric pressure in the middle ear (Densert et al., 1977). Such recordings in man can be made provided that the subject can keep the Eustachian tube open voluntarily during a change in posture as well as in the recumbent position. Very few subjects are able to do so, which makes it more suitable to record the tympanic membrane displacement as induced by a stapedius reflex, since the middle ear pressure can then be equilibrated and checked after a change in posture and before the recordings in recumbency.

When performing such experiments in subjects with a reduced capacity for middle ear pressure equilibration it is possible, however, to balance the middle ear pressure by changing the ambient pressure by means of a pressure chamber Such a balance procedure will place the tympanic membrane in its neutral position. However, the equipment now available only allows a determination of the pressure gradient across the eardrum within 0.1 kPa, a pressure gradient which in some ears affects the quantitative determination of the stapedius reflex response. Experiments are now in progress in order to find a way to determine the quantitative stapedius reflex in all subjects, independently of the Eustachian tubal function.

The results presented in this thesis show that the perilymphatic pressure varies with changes in posture. The intracranial pressure is known to increase by about 1 kPa with a change in posture (Best & Taylor, 1945). Furthermore, it has been shown that the venous pressure on the level of the middle ear increases by about 1 kPa with a change in posture from sitting to recumbency (Ingelstedt et al., 1967, Jonson & Rundcrantz, 1969). If the cochlear aqueduct has a pressure-equalizing function, the intralabyrinthine pressure could be expected to increase when the intracranial pressure is raised. However, since the increase in intracranial pressure is mainly an effect of a hydrostatic pressure increase (Dawson, 1967), it might be assumed that the intralabyrinthine pressure would also increase as a result of the same mechanism, even without any pressure-equilibrating function on the part of the cochlear aqueduct. It therefore seems most probable that the recorded changes in the stapedius reflex response, demonstrated in the present study, are due to a perilymphatic pressure

increase by about 1 kPa

Repeated determination of the quantitative stapedius reflex response have shown a high reproducibility, which makes it possible to compare results from one occasion to the other, and to record changes in the perilymphatic pressure in the future

Interest in inner ear pressure variations has largely been focused on Meniere s disease Today, this disease is considered synonymous with *endolymphatic hydrops, but the etiology is still unknown One question is* whether or not there are pressure changes in the inner ear causing the histopathological feature demonstrated by Hallpike & Cairns (1938), Lindsay (1942), Schuknecht et al (1962), Gussen (1973) and Kimura (1976) as well as the symptoms of Meniere s disease Tonndorf s studies in 1957, however, indicate to some extent that the early fluctuant symptoms of Meniere s disease are induced by changes in the endolymphatic pressure Tonndorf could reproduce the low tone hearing loss by increasing the endolymphatic pressure by means of a mechanical cochlear model, and his findings have later been confirmed in experiments on animals by McCabe & Wolsk (1961)

As it is impossible for obvious reasons to perform recordings of the endolymphatic pressure in man, attempts have been made to find out if it is possible to achieve indications of inner ear pressure variations, assumed to be related to Ménière s disease, by recordings performed on the outside of the inner ear Thus in recent studies on patients with unilateral Ménière s disease, the affected and the unaffected ears have been compared for the purpose of finding out whether or not there are differences between the ears in acoustic impedance Hall & Hughes (1975) and Hall (1978) found differences which they believed to be due to differences in inner ear pressure between the ears, while Herman et al (1977) could not find any differences

In our clinic, research on Meniere s disease has been concentrated on the mechanism underlying the acute attack, and patients suffering from acute symptoms have been exposed to underpressure in a pressure chamber Exposure to underpressure, inducing a relative overpressure in the middle ear, was thought to reduce the endolymphatic pressure (Densert et al , 1975, Ingelstedt et al , 1976, Tjernstrom et al , 1977, Tjernstrom et al , 1979) This idea emerged from earlier studies in our laboratory demonstrating the possibility of affecting the inner ear function by pressure changes in the

middle ear, both indirectly by changing the ambient pressure and directly by inducing an overpressure directly into the middle ear (Ingelstedt, Ivarsson & Tjernstrom, 1974; Tjernstrom, 1974).

It has also been found possible to induce decongestion of the vascular bed of the middle ear by reducing the ambient pressure, provided that the middle ear pressure was not equilibrated (Andréasson, 1973; Andréasson et al., 1976). In view of these findings it was thought possible to induce volume variations of the vascular bed in any closed cavity of the body. As it has been possible to relieve acute symptoms of Ménière's disease and improve low tone hearing by exposure to underpressure, it seems probable that in some way this treatment affects the pressure conditions of the inner ear. This derives some support from the results presented by Tonndorf (1957) and McCabe & Wolsk (1961).

As the results presented in this thesis have shown that it is possible to record variations in the perilymphatic pressure on the outside of the tympanic membrane, it seemed of interest to study whether or not any indications of a change in the perilymphatic pressure could be recorded in patients improved by exposure to underpressure. In a preliminary study, measurements of the quantitative stapedius reflex response have been performed before and after exposure of the patients suffering from acute symptoms to underpressure (Casselbrant et al, 1979). In some patients, who experienced immediate relief of acute symptoms and whose hearing improved, differenc in the reflex response were recorded between the two investigations, which was never seen in normal subjects. We assume that these differences in the reflex response are due to a change in the perilymphatic pressure induced by the exposure to underpressure. However, the possibility of a distended sacculus, which might bulge towards the stapedial footplate (Fick, 1968) and restrict the movement of the footplate mechanically, must be kept in mind

Experiments were also performed on human temporal bones to find an explanation of the outward or inward movement of the tympanic membrane caused by a simulated muscle contraction In 1978, using an electromechanical model, Brask tried to explain the opposite movements by differenc in elasticity between the anterior and posterior poles of the annular ligament. However, present investigations of the temporal bones showed that a simulated stapedius muscle contraction always caused an increase in the perilymphatic space, i e the stapedial footplate was moved towards the middle ear no

matter whether a temporal bone with an outward or an inward movement of the tympanic membrane was investigated.

Photographic documentation showed that a simulated stapedius muscle concentration caused a sliding movement in the incudostapedial joint. The articular surface of the head of the stapes was highly variable in form, which is in accordance with the results presented by Dass et al (1966) and Anson & Donaldsson (1973) Thus, during the sliding movement of the processus lenticularis slid either upwards or downwards, consequently the long process of the incus moved towards or away from the tympanic membrane which moved outwards respectively inwards as the long process of the incus and the tympanic membrane had been shown to move in the same direction.

EXPERIMENTAL STUDIES ON THE STAPEDIUS REFLEX RESPONSE AND THE INFLUENCE OF DIFFERENT PERILYMPHATIC PRESSURES IN HUMAN TEMPORAL BONES

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Abstract

An acoustically elicited stapedius muscle contraction in man has been shown to cause either an outward or an inward displacement of the tympanic membrane. Change of posture affects the displacement and three different patterns of movement have been found. In the present study experiments on human temporal bones were performed. A simulated muscle contraction was elicited by loading the stapedius muscle with weights, and the effect on the displacement of the tympanic membrane caused by applying different pressures to the perilymph was studied. In these experiments it was possible to reproduce all three patterns of stapedius reflex response which had been observed in man as caused by change of posture. The results thus indicate that the different responses found in man and induced by change of posture can be explained by a change in the perilymphatic pressure. It was also observed that the movement of the stapedial footplate caused by a simulated stapedius muscle contraction induced an increase in the volume of the perilymphatic space. Furthermore the movement of the tympanic membrane and the long process of incus always took the same direction.

An acoustically elicited stapedius muscle contraction in man is known to cause either an inward or an outward displacement of the tympanic membrane This has been shown by Mendelson (1966), Lidén et al (1970) and Brask (1978). The methods used by them, however, closed ear canal manometry, did not admit of a quantitative measurement of the displacement. By means of the integrating microflow method devised by Elner et al (1971) it was possible to achieve recordings of the direction of movement as well as a quantitative measurement of the displacement of the tympanic membrane Casselbrant et al (1978) could then demonstrate a quantitative change in the displacement as well as a change of direction induced by a change in posture of the examined subject (1) an outward displacement of the tympanic membrane recorded with the subject in a sitting position was less pronounced when elicited in recumbency or (2) changed to the opposite direction, (3) in ears with an inward displacement recorded in the sitting

position, recumbency increased the response. The only change of the experimental conditions in these experiments was *the change of posture*, which indicated that the same mechanism was responsible for the changed response

In experimental studies on human temporal bones (Densert et al., 1977) it was demonstrated that an increase in the perilymphatic pressure could induce the same pattern of stapedius response change as indicated above by (1) and (2). In that material, however, there was no temporal bone in which the primary response was an inward movement of the tympanic membrane. It is therefore still unknown if an increase of the perilymphatic pressure could also explain the increased response according to the pattern indicated above by (3).

The aim of the present study was to find out

(A) if it is possible to find temporal bones with an inward movement of the tympanic membrane elicited by a simulated stapedius muscle contraction,

(B) if it is possible to reproduce all three patterns of reflex responses in human temporal bones by changing the perilymphatic pressure;

(C) if it is possible to find the mechanical explanation of the different directions of movement of the tympanic membrane.

MATERIAL

Temporal bones taken from adult patients aged between 23-78 years were used for this study. There was no recorded history of ear diseases and microscopic examination showed normal tympanic membranes and middle ears. The middle ear was not opened for inspection before the recordings. The number of temporal bones used will be given separately for each investigation, as many temporal bones had to be excluded owing to the impossibility of applying an overpressure to the perilymphatic space, difficulties in operating technique or middle ear pathology.

The investigations was performed less than 24 hours after death. During the time between the autopsy and examination the temporal bones were stored at +5^{o}C in a humid chamber to avoid change in elasticity due to postmortem changes Ivarsson and Pedersen (1977) reported that the tympanic membrane

system and the mobility of the round and oval windows did not change in 36 hours if the temporal bones were stored in this way.

METHODS

1. Equipment

A stapedius muscle contraction was simulated by loading the stapedius muscle intermittently, with weights of 5, 10 or 15 g, which induced a movement of the tympanic membrane as well as of the stapedius footplate. Quantitative recordings of the volume displacement of the tympanic membrane (ΔVtm_{stap}) and the perilymph (ΔVp_{stap}) were made by using the integrating microflow method devised by Elner et al. (1971).

A flowmeter was connected airtightly with a cuff to the external ear canal (A in Fig.1)or to a fistula fixed with Cyanolit[R] on the superior semicircular canal (C in Fig. 1). To avoid the influence of small variations in temperature and ambient pressure an adjustable reference system operating an identical flowmeter was used, by means of which such influences could be substracted. For further details see Elner et al. (1971) and Casselbrant et al (1977)

Via the fistula on the superior semicircular canal it was also possible to apply a constant overpressure to the perilymphatic system by means of a pressure unit (C in Fig. 1). Recordings of the perilymphatic pressure in relation to ambient pressure were made by a pressure transducer (B in Fig. 1). For further details see Ivarsson & Pedersen (1977).

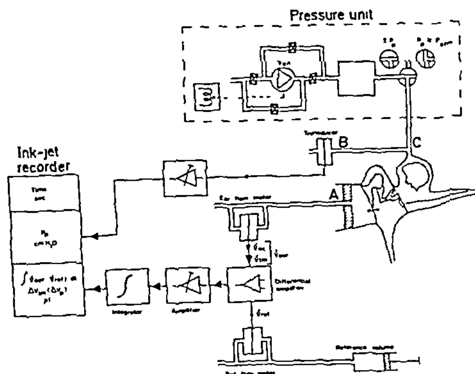


Figure 1 Schematic diagram of integrating microflow and pressure unit.

Symbols Pp Perilymphatic pressure
Vtm Volume displacement of the tympanic membrane in relation to its neutral position
Vp Volume displacement of the perilymph when the flowmeter is connected to C
Δ Before a symbol indicates a change of the variable

II Preparation of the Musculus Stapedius

Mastoidectomy was performed and the facial nerve was explored from the stylomastoid foramen, followed to the second bend and resected. After removal of a usually thin bony septum the M stapedius could be visualized from behind The muscle was then fully dissected About 1/3 of the bony canal of the M stapedius behind the pyramidal eminentia was left intact A thin thread (silk 4 0) was tied around the muscle. The weights were

attached to the thread and repeated loadings were performed At the loadings the thread hung freely in the longitudinal direction of the muscle (see Fig 2) The temporal bones were placed in a position which corresponded to the "sitting position



Figure 2 Musculus stapedius after the preparation

P E R F O R M A N C E A N D R E S U L T S

1 *The volume displacement of the tympanic membrane as a function of different loadings of the stapedius muscle*

Table I presents the volume displacement of the tympanic membrane (ΔVtm_{stap}) caused by loading the stapedius muscle intermittently with weights of 5, 10 or 15 g Eighteen temporal bones were examined and the results are provided as the mean values of five recordings During these examinations the perilymph and the middle ear were in free communication with the atmosphere It appears from the table that the volume displacement of the tympanic membrane increased with heavier weights The direction of the displacement, however, was always the same in each bone It was also possible to record a movement inwards of the tympanic membrane

	ΔVtm_{stap} in µl		
Bone	5 g	10 g	15 g
2	+0.07	+0.15	+0.20
3	+0.06	+0.12	+0.14
6	+0.31	+0.50	+0.65
9	+0.14	+0.20	+0.29
10	+0.07	+0.12	+0.17
11	+0.19	+0.36	+0.42
13	+0.09	+0.13	+0.18
14	+0.08	+0.13	+0.14
27	-0.04	-0.11	-0.14
54	+0.13	+0.21	+0.28
70	+0.12	+0.19	+0.26
71	+0.06	+0.12	+0.20
72	-0 01	-0.03	-0.04
73	+0.04	+0.07	+0.09
74	+0.18	+0.29	+0.41
75	+0.10	+0.18	+0.28
76	+0.07	+0.12	+0.20
78	+0.10	+0.13	+0.14

Table I. The volume displacement of the tympanic membrane (ΔVtm_{stap}) in µl caused by loading musculus stapedius with different weights
+ volume displacement outwards
- volume displacement inwards

II. The effect of different perilymphatic pressure on the Vtm_{stap}

Table II presents the volume displacement of the tympanic membrane (ΔVtm_{stap}) caused by loading the stapedius muscle intermittently with 15 g, which gave the greatest response. The recordings were made with different overpressures of 0.5, 1.0 or 1.5 kPa (5, 10 or 15 cm H_2O) applied to the perilymphatic system. The atmospheric pressure was used as reference. The middle ear was in free communication with the atmosphere during these experiments.

An increase of the perilymphatic pressure caused a quantitative change of the displacement and in one temporal bone the movement of the tympanic membrane changed direction Three different patterns were observed and the results given in Table II represent the mean values of five recordings

ΔVtm_{stap} (15 g) in µl, at different Pp in kPa					
Bone	0	0 5	1 0	1 5	0
2	+0 20	+0 17	+0 16	+0 14	+0 19
9	+0 29	+0 27	+0 25	+0 21	+0 29
13	+0 18	+0 15	+0 12	+0 10	+0 18
14	+0 14	+0 12	+0 10	+0 08	+0 14
28	0 11	0 15	0 18	0 21	0 11
43	+0 02	0 03	0 05	0 08	+0 02
52	0 02	0 05	-0 06	-0 09	-0 02
54	+0 28	+0 25	+0 22	+0 17	+0 28
72	0 04	0 05	0 06	0 07	0 04
73	+0 09	+0 07	+0 04	+0 02	+0 09
74	+0 41	+0 37	+0 35	+0 31	+0 41
76	+0 20	+0 18	+0 16	+0 14	+0 20
78	+0 14	+0 11	+0 09	+0 06	+0 14

Table II The volume displacement of the tympanic membrane (ΔVtm_{stap}) in ul caused by loading musculus stapedius with 15 g when different overpressures are applied to the perilymph (Pp)

1) a pronounced outward displacement of the tympanic membrane was reduced by a perilymphatic pressure increase, (see Fig 3 A) (temporal bones 2, 9 13, 14, 54, 73, 74, 76 and 78)

2) a small outward displacement of the tympanic membrane changed to an inward displacement by a positive perilymphatic pressure (temporal bone 43)

3) an inward displacement of the tympanic membrane was more pronounced when a positive perilymphatic pressure was applied (see Fig 3 B) (temporal bones 28 52 and 72)

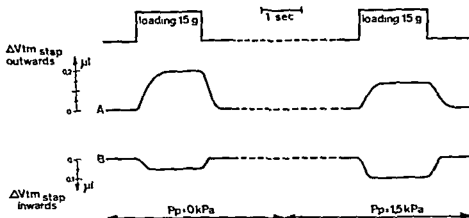


Figure 3 Recordings from two temporal bones, one with an outward (A) and one with an inward (B) movement of the tympanic membrane (ΔVtm_{stap}) in µl caused by loading the musculus stapedius with 15 g, at atmospheric pressure (0 kPa) and after application of pressure (1.5 kPa) to the perilymph (Pp).

III. The volume displacement of the stapedial footplate at a simulated stapedius muscle contraction

The volume displacement of the perilymph (ΔVp_{stap}) is given as an effect of a simulated stapedius muscle contraction (15 g or a cautious pull). During these examinations the middle ear as well as the perilymphatic space were in free communication with the atmosphere For the experiments temporal bones were used with an inward movement (5 temporal bones) and others with an outward movement (9 temporal bones) of the tympanic membrane, as elicited by a simulated stapedius muscle contraction. Although the two different types of temporal bones were used, the recordings always indicated an increase in volume of the perilymphatic space, i.e. the displacement of the perilymph, induced by the movement of the stapedial footplate, was always directed towards the middle ear The displacement of the perilymph was 0 01-0 02 µl

IV The movement direction of the tympanic membrane caused by movement of the long process of the incus

The movement direction of the tympanic membrane was recorded continuously when the long process of the incus was moved very carefully with an operating needle towards or away from the tympanic membrane For this study one temporal bone was selected whose tympanic membrane moved inwards as a

result of a simulated stapedius muscle contraction, and one bone with an outward movement was chosen. The recording showed that the long process of the incus and the tympanic membrane always move in the same direction no matter which temporal bone was investigated.

V. Gravitational force exerted on the ossicular chain at change in posture

The volume displacement of the tympanic membrane (ΔVtm_{stap}) caused by loading the stapedius muscle with a weight of 15 g was recorded (5 temporal bones) when the temporal bones were placed in positions which corresponded to sitting or recumbent positions with the same loading of the stapedius muscle and the same direction of traction in both positions. The middle ear and the perilymphatic space were in free communication with the atmosphere. The recorded response amplitude was the same in spite of the position of the temporal bone. This indicates that the gravitational force exerted on the ossicular chain has no influence on the displacement of the tympanic membrane caused by a simulated stapedius muscle contraction.

DISCUSSION

It appears from these results that there are temporal bones in which a simulated stapedius muscle contraction causes an inward movement of the tympanic membrane. It is probable that the lack of such results in the study by Densert et al. (1977) was due to the limited number of bones examined in their study rather than to their way of loading the stapedius tendon. They applied the weights to the tendon perpendicularly to the axis of traction, whereas the present author devised a technique by which the stapedius muscle was loaded in as "physiological" a way as possible, i e. the weights were applied to the muscle behind the intact pyramidal eminentia, so that a traction in the direction of the tendon was elicited The present author then found the relation between "inward and outward bones" to be 13/65 (16 7 %)

This should be compared with the findings in man by Brask (1978), 15/88 (14.6 %) and by Casselbrant et al (1978) 5/14 (26 4 %). In this study of the effect of different perilymphatic pressures on the simulated stapedius reflex responses, it was possible to reproduce all three patterns of the

stapedius reflex responses that were seen in man as effects of change in posture. It was thus possible to explain all the different responses observed in man, and induced by change in posture, by changes in the perilymphatic pressure.

As it is known that changes in the middle ear pressure affects the stapedius reflex response (Terkildsen,1960; Klockhoff,1961, Djupesland, 1969; Peterson & Lidén, 1972; Renvall & Holmquist, 1976), and as the quantitative effect was shown by Casselbrant et al. (1977), all experiments in the course of the present study were performed while maintaining atmospheric pressure in the middle ear, thus simulating a perfectly equilibrated middle ear pressure.

Keeping the present results in mind, it might be concluded that it is possible to record variations in the perilymphatic pressure outside the tympanic membrane with the integrating microflow method, provided that the middle ear pressure is completely equlibrated.

The conditions for simulating an acoustically elicited stapedius muscle contraction were (1) a "physiological" direction of the traction and (2) a weight load corresponding to the acoustic reflex. The first condition was satisfied as mentioned above and the second as described below. Nergaard et al. (1963) and Cancura (1970)performed investigations on temporal bones in order to find the attenuating effect of the middle ear muscle on sound transmission. They found that a load of 10 g on the stapedius muscle had an attenuating effect of about 8-12 dB at 1 kHz. The investigations made by Borg (1968) on patients with unilateral Bell's palsy showed that a pure tone of 500 Hz (20 dB above the reflex threshold) was attenuated by 12-15 dB, whereas a tone of 1450 Hz (16 dB above the reflex threshold) was attenuated by 0-6 dB. The results of these investigations seem to support the idea that loading the stapedius muscle with 5-15 g corresponds approximately to an acoustically elicited stapedius muscle contraction. As the volume response increased with heavier weights and the movement direction of the tympanic membrane was constant (Table I), a weight of 15 g was used in the other experiments.

The displacement of the stapedial footplate induced by a stapedius muscle contraction has been a topic of discussion for years. Eysell (1870) found that the stapes footplate was placed excentrically in the oval window and the annular ligament was wider at the anterior pole (100 μ) than at the posterior one (15 μ). He also believed that the greatest mobility was

at the anterior pole and that a stapedius muscle contraction pulled the anterior pole towards the middle ear. Dahman (1929), von Bekesy (1936), Brunner (1954), Kobrak (1959), Harty (1963) and Love & Steam (1978) were of the same opinion. Bolz & Lim (1972) demonstrated that most human stapediovestibular joints are incomplete diarthroses; the posterior pole contains an articular cavity, which develops after childhood.

On the other hand, Brask (1978) tried to explain the mechanism causing the outward and inward movement of the tympanic membrane by assuming differences in the elasticity of the anterior and posterior parts of the annular ligament. He thought that with a more pronounced elasticity in the ligament at the anterior pole, a stapedius muscle contraction would cause an outward movement of the tympanic membrane. Furthermore, with greater elasticity at the posterior pole, the stapedius muscle contraction would cause an inward movement of the tympanic membrane as the posterior pole moves towards the vestibulum. He also tried to prove his hypothesis by means of an electromechanical model.

In the present study, however, it was possible to show that a simulated stapedius muscle contraction always caused a displacement of the perilymph towards the middle ear, i.e. there was an increase in the perilymphatic space. This happened independently of an inward or an outward movement of the tympanic membrane. Already in 1867 Politzer demonstrated in experiments on dogs that a stapedius muscle contraction elicited by electrical stimulation of the facial nerve caused decrease in the intralabyrinthine pressure

The linkage between the tympanic membrane and the stapedial footplate makes it difficult to find the mechanical explanation of the opposite movement of the tympanic membrane and the footplate As shown in the present study, the explanation could not be found in the malleoincudal joint and its ligament, as it was demonstrated that the tympanic membrane and the long process of the incus always moved in the same direction. Therefore one should look for the explanation in the incudostapedial joint or in variations of the anatomical relation between the stapes, the pyramidal eminentia and the insertion of the stapedial tendon.

EXPERIMENTAL STUDIES ON THE BIO-MECHANICS OF THE INCUDOSTAPEDIAL JOINT IN HUMAN TEMPORAL BONES

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Abstract

Photographic recordings of the incudostapedial joint in human temporal bones were performed to analyse its movements as caused by a stapedius muscle contraction simulated by loading the muscle with weights The purpose was to find the explanation of the outward or inward movement of the tympanic membrane caused by a stapedius muscle contraction Using the double exposure photo technique, i e one exposure when the stapedius muscle was unloaded and the other when it was loaded, a sliding movement in the incudostapedial joint could be shown Investigations of the articular surfaces of the head of the stapes showed that they were highly variable in shape, but from a functional point of view two different types could be identified Thus, contraction of the stapedius muscle caused a sliding movement in the incudostapedial joint and as a result of the shape of the articular surfaces of the head of the stapes, the processus lenticularis was shown to slide upwards or downwards For this reason the crus longum incudis was moved towards or away from the tympanic membrane,which was displaced outwards respectively inwards, as the tympanic membrane and the crus longum incudis had been shown to move in the same direction

Experimental studies on human temporal bones have shown that independently of the direction of the movement of the tympanic membrane, the movement of the stapedial footplate caused by a simulated stapedius muscle contraction always induces an increase in the perilymphatic space Nor is there any difference in the movement direction of the tympanic membrane and the crus longum incudis, when this is manipulated towards or away from the tympanic membrane (Paper III) For this reason, interest was focused on the incudostapedial joint and the stapedius tendon in order to find the explanation for the outward or inward movement of the tympanic membrane caused by a stapedius muscle contraction

The connection between the incus and the stapes is said to be shaped almost like a ball and-socket joint (Guelke & Keen, 1952, Kobrak, 1959, Harty, 1964) The concave fovea on the head of the stapes forms the socket and has been shown to be very variable in shape (Dass et al , 1966, Anson

& Donaldsson, 1973), and the processus lenticularis of the crus longum forms the ball. The joint capsule is loose and consists mainly of elasti fibres (Davies, 1948, Harty, 1963, Djupesland & Ekeland Gronas, 1973). L the capsule the tendon of the stapedius muscle contains elastic fibres (Davies, 1948, Harty, 1963; Djupesland & Ekeland Gronas, 1973). The tenc extends through the opening of the apex of the eminentia pyramidalis and has usually been considered to insert on the posterior surface of the ne or head of the stapes (Bast & Anson, 1949; Wever & Lawrence, 1954; Kobra 1959, Platzer, 1961; Anson & Donaldsson, 1973; Schuknecht, 1974). Some tendon fibres are also found to mix with the fibres of the capsule of th incudostapedial joint (Politzer,1901; Wolff & Bellucci, 1956; Alberti & Dawes, 1961). However, Djupesland & Ekeland Gronas (1973) found that bet ween 20 and 50 per cent of the tendon fibres of the stapedius muscle wer directly connected to the incudostapedial joint capsule, and several of the tendon fibres even crossed the joint, connecting the stapedius muscl directly with the processus lenticularis.

Studies on cats and rabbits have shown that the contraction of the stape dius muscle causes a sliding movement in the incudostapedial joint (Phil 1932, Kobrak, 1953, Galambos, 1956, Wersall, 1958, Kirikae, 1960, Møller 1964) This is supposed to occur in man but cannot be directly observed since the incudostapedial joint is inaccessible to investigations. Howev when the ossicular chain is interrupted, inspection through a perforatic in the upper quadrant of the tympanic membrane shows that the head of th stapes is pulled posteriorly towards the eminentia pyramidalis.

The aim of the present investigation was to try and find the explanatior of the outward or inward movement of the tympanic membrane by analysing movements of the incudostapedial joint

METHOD AND PERFORMANCE

For this study human temporal bones were used taken from adults with normal tympanic membranes and middle ears. By means of the integrating microflow method the direction of movement of the tympanic membrane caus by a simulated stapedius muscle contraction was recorded on each tempo a bone before further investigations. During the experiments the middle ea and perilymphatic space were in communication with the atmosphere. For

further details see Paper III

In order to make possible inspection and photography via a Zeiss operating microscope,openings were performed in the tegmen tympani and jugular fossa, leaving the tympanic membrane and the ossicular chain intact Small color spots, about 0 5 mm in diameter, were placed on the head of the stapes and on the processus lenticularis so that the movements of the ossiculars caused by the simulated stapedius muscle contraction could be recorded Photos were taken of the incudostapedial joint and the tendon of the stapedius muscle at magnifications of 16x and 25 x with the double exposure photo technique, i e one exposure was made when the stapedius muscle was unloaded and the other when it was loaded This photo technique makes it possible to show if a movement has taken place or not, but the disadvantage is that the direction of this movement cannot be decided with certainty from the photos

In order to investigate the articular surfaces separately the capsule of the incudostapedial joint was opened, and the stapes and the incus were extracted from temporal bones with an outward (6 temporal bones) as well as an inward (6 temporal bones) movement of the tympanic membrane caused by a simulated stapedius muscle contraction When all soft tissue had been removed, the stapes and the incus were photographed using a scanning electron microscope (SEM)

RESULTS

When comparing temporal bones with an outward or an inward movement of the tympanic membrane,respectively, no noticeable difference could be found at microscopic inspection with regard to the tendon of the stapedius muscle and the anatomical relation between the apex of the pyramidal eminentia and the point of insertion of the tendon on the stapes This has been assumed as a working hypothesis in Paper II

Whether there was an outward or an inward movement of the tympanic membrane, the loading of the stapedius muscle in order to simulate a stapedius muscle contraction was shown to cause a sliding movement in the incudostapedial joint, which could be observed under microscopic magnification The stapes made a rotary movement around an axis close to the posterior end of the

stapedial footplate, and the head of the stapes was moved posteriorly in the direction of the stapedius tendon towards the pyramidal eminentia. At the same time the incus made a sliding movement and in some temporal bones the processus lenticularis was seen to move a small distance in the same direction, but the movement of the stapes was always greater. Apart from the sliding movement in the incudostapedial joint, a translatory movement of the processus lenticularis towards or away from the tympanic membrane was also recorded. However, the direction of the movement could not be decided with certainty from the photos. Thus, the differences in the movement of the stapes and the processus lenticularis, recorded at a simulated stapedius muscle contraction, were probably due to the biomechanics of the incudostapedial joint.

The anatomical investigations of the articular surfaces showed that their shapes were highly variable from one specimen to another. From a functional point of view two different types of surfaces could be identified.

In temporal bones with an outward movement of the tympanic membrane the concave surface of the head of the stapes was always deeper and more elliptically shaped and the anterior margin was higher than the central part (see Fig. 1). On the other hand, in temporal bones with an inward movement of the tympanic membrane the articular surface was more shallow and the anterior margin was lower than the central part (see Fig. 2).

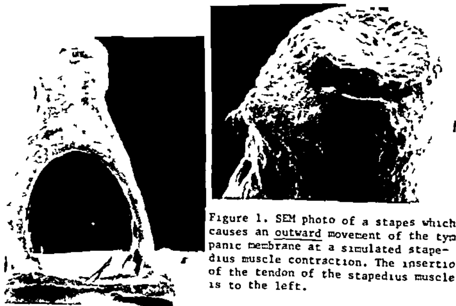


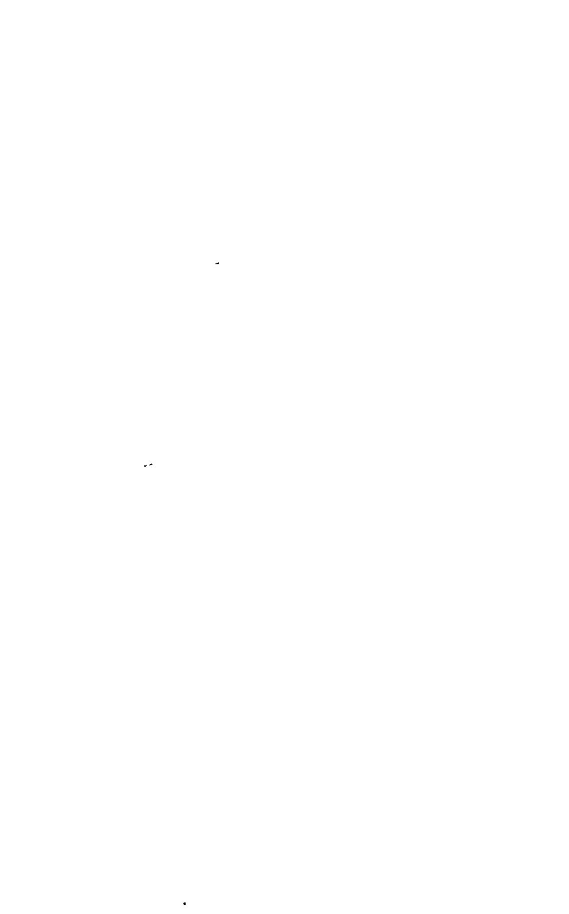
Figure 1. SEM photo of a stapes which causes an outward movement of the tympanic membrane at a simulated stapedius muscle contraction. The insertion of the tendon of the stapedius muscle is to the left.

shown in experiments in both man (Ivarsson, 1975) and temporal bones (Densert et al , 1977) to occupy a more outward position when the perilymphatic pressure is increased Further studies are in progress

SUMMARY

This study was perfomred in order to find methods and procedures for indirect recordings of variations in the perilymphatic pressure By means of a microflow method it was possible to record quantitatively the outward or inward displacement of the tympanic membrane, caused by an acoustically elicited stapedius reflex Since changes in posture were assumed to cause changes in the perilymphatic pressure recordings of the reflex response in sitting and recumbency were made This change in posture was however shown to increase the middle ear pressure by 0 1 kPa, which could be demonstrated to affect the reflex response It was thus necessary to choose subjects for the investigations who could equilibrate their middle ears perfectly When comparing the reflex responses recorded in sitting and recumbency, differences were found which could be referred to three different patterns Furthermore, when studying the effect of different perilymphatic pressures on a simulated stapedius reflex response in human temporal bones, it was possible to reproduce all three patterns of the stapedius reflex response that were seen in man as effects of changes in posture It was thus possible to explain all the three different responses observed in man and induced by change in posture by change in perilymphatic pressure From these results it might be concluded that it is possible to record variations in the perilymphatic pressure outside the tympanic membrane with the microflow method, provided that the middle ear pressure is completely equilibrated

Experimental studies were also performed in temporal bones in order to find the mechanical explanation of the outward or inward movement of the tympanic membrane The stapedius muscle contraction was found to cause a sliding movement in the incudo stapedial joint and due to the variable shape of the articular surface of the head of the stapes, the processus lenticularis slid upwards or downwards, which displaced the tympanic membrane outwards and inwards, respectively



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SUPPLEMENT 363

Fluid Motion in the Mammalian Organ of Corti

A Possible Source of the Second Filter

BY

GLENN H. FROMMER

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ACTA OTO-LARYNGOLOGICA

SUPPLEMENT 363

Fluid Motion in the Mammalian Organ of Corti

A Possible Source of the Second Filter

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This paper is dedicated to
Charles R. Steele
friend and advisor

Abstract

A mechanical analysis of the functioning of the mammalian organ of Corti as deduced from experimental observations and electronmicroscopic studies of the organ has been conducted It was found that the arch of Corti is responsible for initiating a fluid motion in the spiral sulcus and reticular lamina which could stimulate the inner hair cells A three-dimensional linear mathematical model with no arbitrary parameters is proposed to describe the force acting on the inner hair cell cilia as a function of arch displacement Analysis of the model results show that the model (*a*) correctly predicts the form of the neural response for a low frequency square wave of arch motion, (*b*) demonstrates a physiologically reasonable time constant of 245 μsec, (*c*) shows a sharpening of neural stimulation of physiological importance, (*d*) can explain the qualitative difference of neural responses to arch motions of opposite polarity, (*e*) demonstrates a phase difference between outer and inner hair cell stimulation, and (*f*) appears to be chemically and metabolically vulnerable

Introduction

Much information has been amassed on the structure and function of the intricate parts of the mammalian ear Yet there exists a serious gap in our understanding of the process(es) by which the motion of the basilar membrane stimulates the hair cells located in the organ of Corti Comparing various accounts of basilar membrane motion with neural response for acoustic stimulation, Evans and Wilson (1973) have shown that the mechanical response of the basilar membrane is not sharp enough to account for the fine frequency discrimination of the neural data It has been postulated by these researchers that a *second filter* between the basilar membrane motion and the auditory nerves vulnerable to chemical and metabolic influences must exist The most conclusive evidence as to the nature of this filter has been given by Russel & Sellick (1977) Recording intracellularly from the inner hair cells of the guinea pig, they observed that these receptors were as finely tuned as the auditory nerve Thus as the sharp frequency discrimination is not of neural origin, it must be concluded that the sharpening is due to the micromechanics of the organ of Corti To seek an explanation as to how the micromechanics could act as a secondary filtering device, it will be necessary to analyse the interrelation of the individual structures in the organ of Corti to obtain the mechanics of the entire organ

It is generally assumed that the hair cells are stimulated by the relative shearing action between the reticular and tectorial membranes (Fig 1) As the basilar membrane moves up,

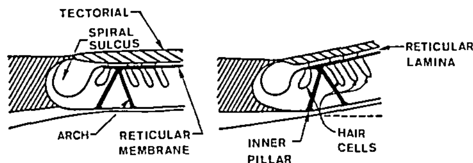


Fig 1 Patter of shearing action between the tectorial membrane and organ of Corti Note the change in cross sectional area as the arch is rotated From Davis (1958)

the arch of Corti rotates about the foot of its inner pillar causing the hair cell bodies located in the organ to move relative to the ends of their cilia which are embedded in the tectorial membrane According to Flock et al (1962), hair cells demonstrate an increase in firing rate when their cilia are bent toward the kinocilium, i e toward the spiral ligament Thus only one-half of a cycle of basilar membrane motion will result in hair cell stimulation, a result observable experimentally

Spoendlin (1967) found in the cat that 60% of the nerves innervating the outer hair cells are efferent, while nerves innervating the inner hair cells are 95% afferent In addition he found that there are approximately 20 nerve endings for each inner hair cell In contrast, there is about one nerve ending for each outer hair cell but with a multiplicity of connections Thus it appears that the inner hair cells are the receptors primarily responsible for the sensing of the motions of the organ of Corti

It has been well substantiated that the longest cilia of the outer hair cells are firmly embedded in small sockets in the tectorial membrane (Kimura 1966, Lim, 1972, Ross, 1974 Hoshino, 1976) There is however, no general consensus regarding the attachment of the inner hair cell cilia to the tectorial membrane Engstrom et al (1962) Kimura (1966), and Lim (1972) found no attachment, while Ross (1974) and Hoshino (1976) found some evidence of a weak attachment in some mammals Nonetheless from these results it can be stated that if there is an attachment of the inner hair cell cilia to the tectorial membrane it must be quite weak in comparison with the firm attachment of the outer hair cilia

If the inner hair cells are the primary motion detectors, and if they are only very loosely attached to the tectorial membrane (or not at all), what force could move these cilia? Further analysis of Fig 1 provides a possible answer As the basilar membrane moves up, the arch will rotate into the fluid filled spiral sulcus, probably causing a decrease in the cross-sectional area of the sulcus This decrease in area would cause a quantity of fluid in the sulcus to move through the sulcus and out the reticular lamina Thus the force exerted by the motion of the fluid in the lamina may provide the stimulus for the inner hair cells

There are experimental observations to support the idea that fluid motion in the reticular lamina exists and is important Zwicker (1972), observed a strong streaming of fluid emanating from the reticular lamina of a pig under a pure tone acoustic stimulation of the cochlea at an intensity of about 100 dB SPL Though this result is for high sound intensities, Zwicker's observation substantiates the idea of fluid motion in the reticular lamina

In this paper, a one-dimensional mathematical model, consistent with the anatomy of the organ of Corti will be utilized to illustrate how arch motion may result in a fluid motion in the lamina acting on the cilia The one dimensional model can be extended to three dimensions by considering fluid motion and continuity in the sulcus The fluid motion out of the lamina predicted by the model is examined for a variety of possible arch motions, and these results are compared with experimental data collected by others

Model of the Organ of Corti

In modeling of any complex physical system it is of utmost importance to first consider the forces acting on the system for it will be the nature of these forces that will determine the type of assumptions that may be utilized to simplify the mechanics of the system From mer & Steele (1979) have shown that the organ of Corti lies a distance above the basilar membrane where the forces exerted by the fluid in and surrounding the organ are primarily due to the viscous nature of the fluid This means that the inertial effects of the motions of the fluid in and around the organ are negligible and we need only consider the steady state motions of the constituents of the organ and its fluid in a mechanical analysis

From the one dimensional representation of the organ of Corti shown in Fig 1 the mechanics of the organ can be ascertained A movement of the basilar membrane upwards toward the scala vestibuli will cause the arch of Corti to compress the inner hair cell border cell area and reduce the cross sectional area of the sulcus The fluid in the sulcus will then be forced out of the reticular lamina thereby stimulating the inner hair cells Mechanically the arch acts as a piston of height h_1 pressing on a mass of cells which will behave as a spring with a spring constant K The fluid in the sulcus at a pressure p_1 will then be forced out along a straight walled channel of height h_2 and length L For simplicity the inner hair cells can be considered stationary located at the opening of the reticular lamina bordering the sulcus Thus a one dimensional mechanical model of the organ of Corti can be conceptualized as shown in Fig 2 where F is the force per unit length acting on the piston IHC denotes the location of the inner hair cells and x_1 and x_2 indicate respectively the direction of positive motion of the arch and the fluid in the reticular lamina

At present there is no direct quantitative measurement of the value of the spring constant K Nonetheless the magnitude and nature of the spring constant can be calculated from information available in the current literature Békesy (1960) examined the stiffness of the organ of Corti with the application of lateral forces to the border inner hair cell area of the sulcus and to Hensens cells He noted that the organ is much stiffer with respect to radial displacement in the sulcus than on the side of Hensens cells This observation is supported by Taber (1976) who examined the effect of geometrical and mechanical properties of the outer hair cell cilia in a model of the organ of Corti In doing so Taber calculated a value of the spring constant K per unit length as $K = 1\ 65 \times 10^5$ dynes/mm*

By considering the mechanical properties of the sulcus wall for small amplitude arch motions the sulcus can be approximated as a long fluid filled tube with rigid walls Because the sulcus walls are rigid motions of the basilar membrane will impart motions to the fluid in the reticular lamina with no time delay

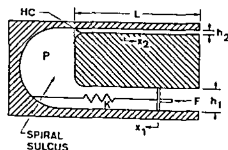


Fig 2 The one-d mensional model of the organ of Corti For d mens ons see Table II

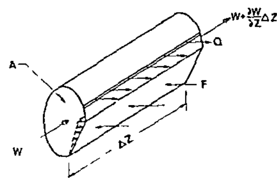


Fig. 3 A picture of a small volume element of fluid in the spiral sulcus illustrating fluid continuity

The reticular lamina is considered as the fluid filled space between the tectorial and reticular membranes Bekésy (1960) noted that while the reticular membrane was stiff concerning deformations, the tectorial membrane behaved as a viscoelastic mass, soft to slow static deformations, but rigid to vibrations at 200 Hz Thus considering the sinusoidal fluid motion in the organ of Corti, the reticular lamina can be conceptualized as a stiff, straight-walled channel whose height is much smaller than its length There are four rows of obstructions in the lamina, one row of flat inner hair cell cilia bundles and three rows of W shaped outer hair cell cilia bundles It is well known in fluid mechanics that an obstruction to fluid motion in a channel acts to decrease the permeability of the channel Similarly an increase in the length of a channel also decreases the channel permeability Therefore a lamina with no obstructions to fluid flow stretched by an amount corresponding to the change in permeability caused by the obstructions can in principal replace the original lamina

Frommer & Steele (1979) have measured in a model experiment and have calculated analytically the permeability of three rows of W shaped cilia bundles and a single row of flat cilia bundles for varying fluid velocities through the row(s) The permeability was defined as a nondimensional fluid velocity divided by a nondimensional pressure drop through the row(s) For fluid velocities less than 0 1 cm/sec, the experimental results indicate values of constant permeability, 0 12 for the three rows, and 0 7 for a single row While the magnitude of sound pressure level corresponding to a fluid velocity of 0 1 cm/sec cannot be determined precisely, it is clear the experimental values of permeability are applicable for fluid velocities several orders of magnitude less than 0 1 cm/sec, i e small amplitude arch motions Thus considering the row of inner hair cell cilia to be 7 μm wide and the outer three rows together to be 25 μm wide, a lamina 130 μm long with the four rows of obstructions will have the same permeability to fluid motion as a lamina 348 μm long with no obstructions (Distances from Tiedemann, 1970 and Thalmann, 1970) In subsequent mention of the length of the reticular lamina in this paper, the length referred to will be that of the stretched lamina

With the mechanics of the one dimensional model understood, the equations of motion for the arch and the fluid in the lamina can be found Assuming the motion of the piston to be controlled by a spring, the displacement of the piston x_1, is defined by

$$x_1 = \frac{F-p_1 h_1}{K} \quad (1)$$

For a viscous fluid moving in a thin straight walled channel, the velocity of the fluid, $\partial x_2/\partial t$, is related to the pressure acting on the fluid by the physical parameters of the channel and the fluid The relationship is given by Flugge (1960) as

$$\frac{\partial x_2}{\partial t} = \frac{h_2^2}{12\bar{\mu}}\frac{p_1}{L} \quad (2)$$

where $\bar{\mu}$ is the viscosity of the fluid

To extend the one dimensional model to three dimensions, it is necessary to understand the functioning of the spiral sulcus The sulcus is a fluid filled tube whose length is about 300 times its width at any point along

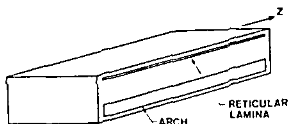


Fig 4 The three-dimensional model of the organ of Corti

the cochlear duct Therefore, at any position along the sulcus away from its ends, the sulcus can be considered as an infinite tube Fig 3 shows a small volume element of the sulcus, with the border cells comprising the straight part of the sulcus wall Under a force, the arch will change the cross-sectional area of the sulcus If A and w represent the cross sectional area of the sulcus and a unit displacement respectively, the continuity of the fluid contained in the volume element can be described as

$$A\frac{\partial w}{\partial z} - x_1 h_1 - Q \tag{3}$$

where z is the direction down the sulcus and Q is the volume flow out of the sulcus defined as $Q = x_2 h_2$

Finally, if the sulcus can be assumed to have a circular cross-section of radius a the fluid velocity in the sulcus can be related to the pressure gradient in the sulcus from Schlichting (1966) by

$$\frac{\partial w}{\partial t} = \frac{a^2}{8\bar{\mu}}\frac{\partial p_1}{\partial z} \tag{4}$$

Eqs (1) through (4) represent the mathematical description of the three dimensional model depicted in Fig 4, with a cross-section as shown by Fig 2 These four equations can be combined to yield a partial differential for the displacement of the fluid in the lamina under the effect of a forcing function The equation is

$$-\left(\frac{3}{2}\frac{Aa^2L}{h_2^3}\right)\frac{\partial^2 x_2}{\partial z^2} + \left(\frac{12\mu L h_1^2}{K h_2^3}\right)\frac{\partial x_2}{\partial t} + x_2 - \frac{h_1}{K h_2}F \tag{5}$$

The forcing function on the right hand side of Eq (5) is multiplied by a geometrical factor h_1/h_2 For normal mammalian cochlea $h_1/h_2 \sim 30$ (Tiedemann 1970) This means that the pressure in the sulcus will be an order of magnitude greater than the pressure in the scala media Therefore the pressure in the scala media can be considered as a constant and we need not consider any phase differences between the pressures in the sulcus and scala media as effecting the fluid motion In addition, because $h_1/h_2 \approx 30$, the velocity of the fluid in the lamina will be about 30 times the velocity of the arch For a first approximation the inner hair cells located between the arch and the sulcus in the organ, can be considered at rest with respect to the piston of the model This substantiates the positioning of the inner hair cell cilia as shown in Fig 2 It is interesting to note the order of magnitude increase in the velocity of the fluid emanating from the lamina over the arch and basilar membrane

By considering a new independent variable ξ as

$$\xi = \left(\frac{t}{T} - \frac{z}{l}\right) \tag{6*}$$

where

$$l = \left(\frac{3}{2}\frac{Aa^2L}{h_2^3}\right)^{\frac{1}{2}} \quad T - \left(\frac{12\bar{\mu}L h_1^2}{K h_2^3}\right) \tag{7}$$

Eq (5) can be reduced to an ordinary differential equation In terms of dimensionless parameters, Eq (5) becomes

$$-\frac{d^2 p}{d\xi^2} + \frac{dp}{d\xi} + p = \mathcal{F}(\xi) \tag{8}$$

It will be somewhat more convenient to consider solutions of Eq (8) for the net volume flow out of the lamina, $q(\xi)$, where

* Though eq (5) is a parabolic equation the substitution eq (6) used for hyperbolic equations is permissible, considering the frequency range and sound pressure levels used in this study

$$q(\xi) = \int p(\xi)\, d\xi \qquad (9)$$

In viscous flow theory, it is known that the force on a body is proportional to the velocity of the fluid impinging upon the body (Stokes approximation). For the flow of viscous fluid in a channel, Eq (2), the velocity of the fluid is proportional to the pressure exerted on the fluid. From Eq (9) it is noted that the pressure is proportional to the derivative of the net volume flow. Therefore, the force exerted by the fluid moving in the reticular lamina will be proportional to the derivative of the net volume ≈ the pressure in the sulcus.

Results of the Model

Solutions of $p(\xi)$ for different $\mathcal{F}(\xi)$ are obtained by first solving Eq (8) for $q(\xi)$. $p(\xi)$ is then found by differentiating $q(\xi)$. An analytical method for this procedure is illustrated below for a unit step forcing function

$$\mathcal{F}(\xi) = \begin{cases} 0 & \xi < 0 \\ 1 & \xi > 0 \end{cases} \qquad (10)$$

Solving Eq (8) for $q(\xi)$ we obtain

$$q(\xi) = \begin{cases} C_1 e^{2\xi} + C_3 & \xi < 0 \\ C_2 e^{-\xi} + C_4 & \xi > 0 \end{cases} \qquad (11)$$

Differentiation of Eq (11) results in the solution for $p(\xi)$ as

$$p(\xi) = \begin{cases} 2C_1 e^{2\xi} & \xi < 0 \\ C_2 e^{-\xi} & \xi > 0 \end{cases} \qquad (12)$$

Constants C_3 and C_4 can be found by evaluating $q(\xi)$ at $\xi = 0$ for a specific step size. C_1 and C_2 are then found from the continuity conditions of $p(\xi)$ and $q(\xi)$ at $\xi = 0$.

The unit step forcing function and its solutions for $p(\xi)$ and $q(\xi)$ can be combined linearly to produce some rather interesting results. In Fig 5, several step functions have been used to construct a square pulse. The corresponding net volume flow $q(\xi)$ and the force exerted by the fluid at the location IHC $p(\xi)$ is shown. A positive value of $\mathcal{F}(\xi)$ corresponds to a displacement of the piston into the sulcus, i.e. a displacement of the basilar membrane toward the scala vestibuli. Similarly, a negative value of $\mathcal{F}(\xi)$ refers to the displacement of the basilar membrane toward the scala tympani. For any change in the magnitude of $\mathcal{F}(\xi)$ there is a corresponding change in $q(\xi)$ and $p(\xi)$. Thus a positive change in $\mathcal{F}(\xi)$, (from − to +) creates in the sulcus a high pressure which is relieved by a flow of fluid through the lamina, $q(\xi) > 0$, and a flow of fluid down the sulcus. A negative change in $\mathcal{F}(\xi)$ creates in the sulcus a vacuum which draws fluid into the lamina, $q(\xi) < 0$. Considering the directionality property of hair cells as demonstrated by Flock et al (1962), we would expect an outflow of fluid to produce a stimulation of the inner hair cells, while an inflow of fluid will produce no stimulation. Therefore, the form of the stimulation presented to the inner hair cells in terms of the model parameters is $p(\xi) > 0$. Thus during one cycle of arch motion, there will be stimulation presented to the inner hair cells over one-half cycle.

The square pulse indicated as $\mathcal{F}(\xi)$ in Fig 5 is actually a traveling wave moving down the length of the model. We can, however, fix our position arbitrarily at any point along the model. Thus $\mathcal{F}(\xi)$ and hence $p(\xi)$ will represent the force on the arch and the predicted stimulation of the inner hair cells at our position as a function of time. In the cochlea, if we pick any single nerve fiber, we have determined a position along the cochlea duct. If

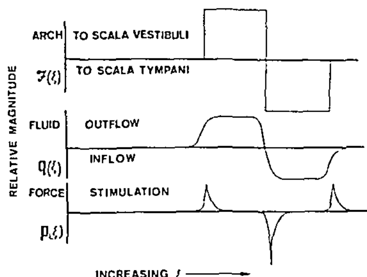


Fig 5 Plot of $\mathcal{J}(\xi)$ and $p(\xi)$ for a square wave of arch motion Redrawn from computer plot

the stimulation presented to the hair cells is slowly varying we would expect the neural response of the fiber attached to that cell to reflect the stimulation Therefore, for a low frequency square pulse as shown in Fig 5, *we would expect the neural response of our* arbitrarily chosen fiber to be similar in form to $p(\xi)>0$

It is time consuming to solve Eq (8) analytically for $q(\xi)$ and $p(\xi)$ for various forcing functions However, with the aid of a computer program developed by S Boel Pedersen, it is possible to examine the effect of a multitude of different types of forcing functions very easily Fig 6, redrawn from a computer plot, shows the forcing function as an impulse with the stimulation $p(\xi)$ Note the sharp rise in the stimulation for $p(\xi)>0$ and relatively slower decay in the stimulation The magnitude of the decay of the stimulus is the time constant of the model, T, as defined by Eq (7) Utilizing distances measured from electronmicroscopic studies presented in Table I, and assuming that the fluid in the organ is *mechanically similar to water*, an approximate value of T can be calculated as

$$T=245\ \mu\text{sec} \tag{13}$$

It is known from the work of Bekesy (1960) that tones producing a sinusoidal variation of pressure at the eardrum produce a displacement of the basilar membrane in the form of a

Table I *Values of the geometrical parameters of the model defined in Fig 2 h_1 h_2 L and a used in the calculation of the time constant T*

Parameter	Magnitude	Animal	Reference page
h_1	75 µm	Human	Thalmann (1970) 1623
h_2	4 µm	Monkey	Kimura (1966) 57
L	130 µm	Human	Thalmann (1970) 1623
a	35 µm	Human	Thalmann (1970) 1623

Unstretched dimension

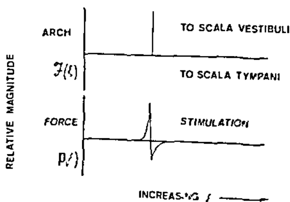


Fig 6 Plot of $\mathcal{J}(\xi)$ and $p(\xi)$ for an impulse in arch motion Redrawn from computer plot

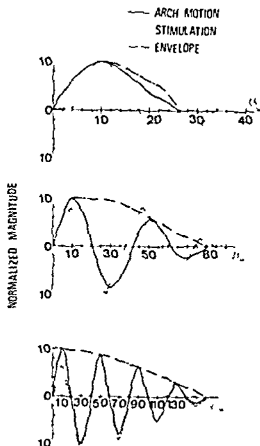


Fig 7 Plots of the arch motion and subsequent stimulation for the model (*a*) (*b*) and (*c*) correspond to arch motions whose wavelengths are 2 1/2 and 1/4 the length of the envelope respectively Redrawn from a computer plot

traveling wave, moving from the stapes to the helicotrema A traveling wave can be considered as a sinusoidal wave, the carrier, enclosed in an amplitude envelope The general shape of the amplitude envelope along the cochlear duct as a function of frequency is fairly well known, and has been measured by Békesy (1960) and several other investigators If the number of wavelengths of the carrier can be calculated per length of the amplitude envelope, than the mechanics of the organ of Corti can be viewed in terms of an arch motion whose period is a fraction of the length of the envelope This will greatly simplify the analysis of the model

The number of wavelengths per traveling wave of various frequency tones can be found if both the speed of wave propagation and the length of the amplitude envelope are known The propagation time for traveling waves produced by a click impulse at a point 25 mm from the stapes was measured by Békesy (1960) for the human He found it to be 1×10^{-3}, sec Dallos et al (1971) have shown this value to be independent of frequency Therefore the speed of all waves is about 2.5×10^{4} mm/sec Békésy also measured the magnitude of the amplitude envelope of basilar membrane displacement in the human along the cochlear duct The length of the envelope for tones of 200, 800 and 1600 cycles per second are approximately 32, 30 and 24 mm respectively (The choice of the length of the full envelope will allow comparison to physiological results presented in the following section of this paper) Therefore, the approximate number of carrier waves per length of envelope for these frequencies is 1 61, 6 04 and 9 64 Upon plotting these values, on a regular scale, as a function of the frequency, on a logarithmic scale, it was discovered that the number of carrier waves is linearly proportional to the frequency initiating those waves These results indicate a tone of 7000 Hz will have 7 55 carrier waves in its envelope

Though these calculations are only approximate, it can nonetheless be concluded that low frequency tones produce traveling waves whose carrier wave exhibits a wavelength of similar magnitude to the length of the traveling wave, while high frequency tones will produce carrier waves whose wavelength is much smaller than the length of the traveling wave

Following this analysis, an amplitude envelope of arch motion for the model was chosen of the form cos ξ and a length $\pi/4$ The wavelength of the arch motion was selected as 2 0, 0 5 and 0 25 the length of the envelope The normalized amplitude of the arch motion and subsequent stimulation is plotted as a function of ξ/ξ_0 in Fig 7*a* *b*, *c* ξ_0 represents the ξ distance from the start of the arch motion to its first maximum The most striking result of Fig 7 is that in all cases, at a normalized amplitude of 1/3, the width of the stimulation

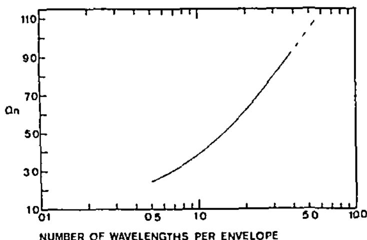


Fig. 8 The value of Qn as a function of the number of wavelengths per length of amplitude envelope

is a fraction of the width of the arch motion, and therefore even a smaller fraction than the width of the envelope of arch motion. A measure of this sharpening can be obtained by considering a parameter Qn defined as the width of the envelope divided by the width of the first area of stimulation, where both widths are measured at a normalized amplitude of 1/3. Fig. 8 shows the variation of Qn as a function of the number of cycles per amplitude envelope for the model. With the information obtained from the calculations of the number of waves per envelope as a function of frequency for the ear, Qn can be directly related to frequency as shown in Fig. 9. The model shows an increasing sharpening with increasing frequency, and values of $Qn = 2.5$ at 1 036 Hz and $Q_n = 12.5$ at 7 000 Hz. Because data utilized in the calculation of waves per envelope is lacking below 1 000 Hz, no attempt has been made to extend the model results into this region.

The relative sharpening of the force exerted by the fluid moving in the reticular lamina compared with the amplitude envelope was not unexpected. Steele (1973) showed the

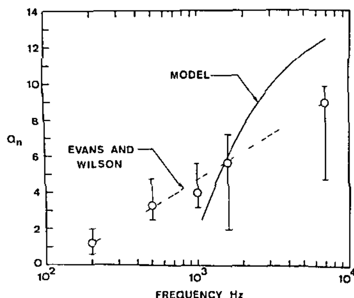


Fig. 9 The value of Qn as a function of frequency for both the model and the experimental results of Evans & Wilson (1973). There are no model results for frequencies less than 1000 Hz.

possibility of such a sharpening in a more idealized mathematical model Steele considered a line of doublets to represent the fluid motion in the sulcus A distribution of doublet strengths along this line was chosen to represent a basilar membrane amplitude curve Calculating the fluid velocity produced by such a distribution indicated a sharpening similar to what is presented in this paper

In addition to the sharpening, the results presented in Fig 7 can be used to examine the stimulations presented to the inner hair cells by arch motions of opposite polarity The stimulation predicted for the arch motion in Fig 7*b* consists of two maxima of similar amplitude followed by a third, smaller maximum If we now consider $-\mathcal{J}(\xi)$ of Fig 7*b* as the arch motion, we must consider $-p(\xi)$ as the *predicted* stimulation Where before there were two peaks of similar magnitude in the stimulation, there is now a large peak followed by one of much smaller amplitude Thus the linear model is capable of producing a seemingly non linear' result A possible physiological interpretation of this result will be discussed in the next section

It has been shown that the model presented herein suggests two different modes of stimulation to the hair cells in the organ of Corti, a result postulated by Dallos (1973) and others from experimental evidence Considering a one dimensional slice of the organ the outer hair cells will be stimulated by the relative shearing motion between the tectorial and reticular membranes while the inner hair cells will be stimulated by the fluid motion caused by the arch changing the cross sectional area of the sulcus Because the reticular membrane is rigidly connected to the top of the arch the stimulation of the outer hair cells will always be *in phase* with the arch motion i e a movement of the basilar membrane upwards, toward the scala vestibuli will cause stimulation However, because the inner hair cells are responsive to the force exerted upon their cilia and this force is in phase with the fluid velocity, we would expect the stimulation of the inner hair cells must lead that of the outer hair cells by 90° This model result agrees precisely with the experimental evidence of Dallos (1972) and Zwislocki & Sokolich (1973)

Considering the three dimensional model the inner hair cell stimulation will always be displaced more apically than the outer hair cell stimulation Furthermore the amount of displacement will be a function of frequency Thus the frequency of arch motion can be considered as being encoded by two mechanisms in the model as a specific place of maximum inner hair cell stimulation, and as a specific place of maximum outer hair cell stimulation

The preceding analysis has considered only the functioning of a normal organ of Corti Much can be learned from an organ in an altered state Tonndorf et al (1962) noted that the tectorial membrane shrank and became more viscous when fluid from the scala tympani, the perilymph, was injected into the scala media This same contraction was discussed by Lim (1972) in conjunction with the effects of fixation on the tectorial membrane It is evident from these results that the physical properties of the tectorial membrane are very susceptible to chemical changes in the fluid surrounding the membrane, the endolymph Investigations of the attachment of the inner hair cell cilia to the tectorial membrane suggest that the ends of these cilia are either very close to, or are lightly touching the underside of the membrane If the membrane were to shrink, it is possible that the contracted tectorial membrane could come into definite contact with the inner hair cell cilia A photograph published by Tonndorf (1974) seems to substantiate this idea Here the cochlear partition has been displaced by a hydrostatic pressure and fixated The photograph shows the inner hair cell cilia clearly touching the underside of the contracted tectorial membrane In this state the force moving the inner hair cell cilia would be a consequence of the relative shearing motion between the reticular and tectorial membranes, and not of the fluid moving through the lamina Thus the sharpening

would be lost In addition, because the force exerted by the shearing motion is an order of magnitude less than the force exerted by the moving fluid, a greater force must now be exerted by the arch to produce the same amount of stimulation, a consequence of Eq (5) Therefore, by slightly altering the chemical composition of the endolymph, we could expect to see an elimination of sharpening and a raising of threshold by h_1/h_2, about 30 dB

For the sake of completeness, attention should be focused on the model behavior as a function of increasing sound pressure level (SPL) This analysis assumes a sulcus with rigid walls and a constant radius Therefore the model can only be applied to small amplitude arch motions probably no greater than 40–50 dB SPL However, within this range the model behavior is linear Higher amplitude SPL necessitates the consideration of a sulcus radius that is some function of sulcus pressure

In addition to changes in the equations defined by the model, it will be very interesting to consider the changes in the amplitude of arch motion along the basilar membrane as a function of SPL with respect to some reference level of amplitude This implies that larger sections of the membrane (with respect to that reference level) will be set into motion as the SPL increases Therefore, a larger area of fluid outflow from the sulcus and hence stimulation is expected This analysis as well as the consideration of a pressure variant sulcus radius is currently being investigated

Comparison of Model and Experimental Results

A one dimensional model of the organ of Corti was derived from previous anatomical investigations of the organ An analysis of the motion of the arch of Corti showed that a movement of the arch into the spiral sulcus produced an emission of fluid out of the reticular lamina The force exerted by the fluid moving through the reticular lamina would then stimulate the inner hair cells Consideration of the fluid continuity in the spiral sulcus extended the one dimensional model to a model of three dimensions The stimulations predicted by the three dimensional model for step impulsive and sinusoidal arch motions were examined It was found that the model (*a*) predicts a specific form of neural response for a low frequency square wave of arch motion, (*b*) demonstrates a time constant estimated for the human at 245 μsec, (*c*) a sharpening of stimulation relative to the amplitude envelope The ratio of the widths of the stimulation and the amplitude envelope increasing for increasing frequencies, from 2 5 at 1036 Hz to 12 5 at 7000 Hz, (*d*) a qualitative change in stimulation pattern with regard to a change in the direction of arch motion, (*e*) predicts phase differences between the stimulations presented to the inner and outer hair cells and (*f*) appears to be chemically vulnerable

(a) Response to a square wave

Sokolich et al (1976) examined single fiber responses produced by low frequency trapezoidal motions of the basilar membrane in gerbils whose outer hair cell cilia had been removed by ototoxic drugs Though trapezoidal and square waves differ in the slope of their rise and fall times, a qualitative comparison of the individual fiber response and predicted response is nonetheless permissible A portion of the results of Sokolich et al are presented in Fig 10 The responses of fibers K2–13 and K4–1 are strikingly similar to the model stimulation in Fig 5 for $p(\xi)>0$ The response of fiber K3–1, though similar to the $p(\xi)>0$ curve, differs from that curve by an

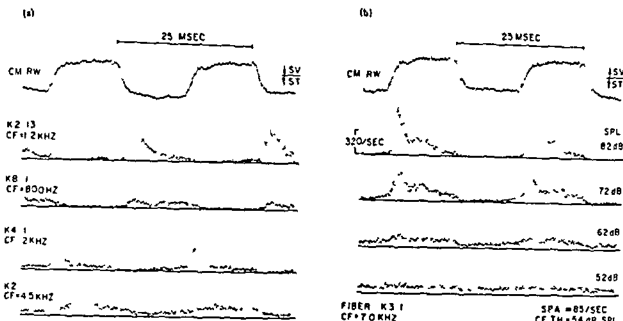


Fig 10 Single fiber responses produced by trapezoidal wave pattern kanamycin treated gerbils (*a*) Upper record shows the averaged round window electrical potential with suppressed whole nerve responses and basilar membrane deflections toward the scala vestibuli and the scala tympani Lower records are histograms showing unipolar responses of four fibers Fibers K2-13 and K8-1 undergo an increase in firing rate when the motion of the basilar membrane is toward the scala tympani while fibers K4-1 and K2-11 illustrate an increase in firing rate when the basilar membrane is displaced toward the scala vestibuli (*b*) Intensity series for fiber K3-1 demonstrates an increase in firing rate when the basilar membrane is displaced toward the scala vestibul (From Sokolich et al 1976)

additional response following the maximum response of the fiber Fibers K3-1 and K4-1 both demonstrate excitation when the basilar membrane is displaced toward the scala vestibuli, a result correctly predicted by the model Unfortunately the response of fiber K2-13 contradicts the model prediction, as the fiber shows excitation when the basilar membrane is displaced toward the scala tympani It is conceivable that the discrepancy between the model prediction and the experimental results is due to a change in functioning of the organ of Corti following the removal of the outer hair cell cilia However it appears that the model is generally capable of predicting the neural response of a single fiber to a low frequency square type pulse

(b) Time constant

There are at present no direct measurements of the mechanical response of the cochlear partition to an impulse stimulus Therefore the model results cannot be tested However it is possible to compare the model prediction to an order of magnitude estimation of the time constant

Utilizing the inverse of the hearing sensitivity curve, Brüel (1975) calculates an averaging time or time constant for the human between 30 and 100 μsec Using nominal dimensions for the organ of Corti, Eq (13) illustrates a time constant of 245 μsec Clearly the model prediction is very close to the value calculated by Bruel If we assume that Bruel s estimate of time constant is accurate to within one order of magnitude, we must conclude the following the mechanism of fluid motion presented by the model is a physiologically reasonable mechanism

Inspection of Eq (7) illustrates the dependence of the time constant on the dimensions of the organ of Corti Tiedemann (1970) has measured the dimensions of the organ of Corti along the length of the basilar membrane A

Table II *Dimensions of the model parameters from Fig. 2 as measured by Tiedemann (1970)*
The time constant is calculated from Eq. (7)

Parameter							Unit
Dist. B.M.[b]	10	19	25	30	33	36	mm
h_1	36	53	63	70	72	75	μm
h_2	2.9	2.2	2.1	2.0	1.9	1.8	μm
L[a]	0.08	0.16	0.21	0.25	0.28	0.30	mm
T	127	798	1 467	2 293	3 011	3 997	μsec

[a] The length represents the unstretched lamina
[b] *This is the distance along the cochlear duct measured from the stapes*

portion of his data as well as the time constant calculated from these dimensions by Eq. (7) are presented in Table II. The model portrays a time constant varying from 127 μsec at a position 10 mm from the stapes, to 3 997 μsec at a position 36 mm from the stapes. Thus, from a consideration of the anatomical data, the model illustrates a time constant whose magnitude decreases with increasing frequency. It will be interesting to see if this prediction can be verified experimentally.

(c) Sharpening

The most important result of the predicted stimulation of the model due to a sinusoidal arch motion, is the existence of a stimulation that is sharper than the applied force. The width of the first area of stimulation divided by the width of the arch amplitude envelope measured at 1/3 the normalized amplitude, as a function of frequency has been presented in Fig. 9. A value of 1/3 normalized amplitude corresponds to a decrease in 10 dB in intensity of arch motion and stimulation. Evans & Wilson (1973) present a plot of the characteristic frequency divided by the bandwidth at a point 10 dB above threshold Q_{10}, for both the basilar membrane and cochlear nerve data. The Q_{10} data for the basilar membrane appears to represent the sharpening of the amplitude envelope while the Q_{10} data for the nerves can be likened to the first area of the predicted stimulation. Thus according to the definition of Q dividing the Q_{10} of the neural data by that of the basilar membrane will render Evans & Wilson's data in a form that can be compared to the model prediction. This has been accomplished and presented in Fig. 9. Also included in this figure is a measure of the scatter of Evans & Wilson's data at 200 through 7 000 Hz. The model prediction compares fairly well to the experimental results in its slope and magnitude above 1 000 Hz. The sharpening for both the experimental results and the model prediction increases with increasing frequency. However, the model does overestimate the magnitude of sharpening by about 30% at 7 000 Hz. Russell & Sellick (1977) recording intracellularly from inner hair cells in the apical portion of the cochlea of the guinea pig, recorded a Q_{10} twice as large as those of Evans & Wilson. Thus even though the method in which the model results were made to exhibit a frequency dependence was only an approximate one, it can be concluded that the relative sharpening predicted by the model is large enough to account for the observable sharpening of the neural output.

(d) Change in stimulation pattern for a change in arch motion polarity

Kiang (1965), in his study of the discharge patterns of single fibers in the cat, noted a qualitatively different response pattern in PST histograms for clicks of opposite polarity. A rarefaction click, one that caused an initial motion of the eardrum outwards, produced a response that contained a single large peak flanked by smaller peaks. A condensation

click, 180° out of phase with the rarefaction click, produced a response that contained two peaks of equal size In addition, the maxima in the response due to the rarefaction click occurred at the minima in the response due to the condensation click

As was explained previously, a change in the polarity of the arch motion of the model produces a change in the predicted stimulation pattern An initial motion of the arch into the sulcus will produce a stimulation pattern with peaks of similar magnitude, while an initial arch motion out of the sulcus will produce a stimulation pattern with only one large peak followed by a smaller one In addition, because of the linearity of the model, the maxima in stimulation from one direction of arch motion will coincide with the minima in the opposite direction of arch motion The response pattern of a single fiber illustrated in a PST histogram can be considered proportional to the average fiber response Furthermore, this average response should be proportional to the stimulation of the receptor cell corresponding to that fiber at low stimulation intensities Therefore, the response pattern of a single fiber in a PST histogram can be likened to the stimulation as predicted by the model It thus appears that the results of the model can be utilized to qualitatively explain the discharge pattern in single fibers due to opposite polarity clicks without introducing any nonlinearities

(e) **Phase differences between the stimulations presented to the inner and outer hair cells**

Zwislocki (1975) has shown analytically that his recordings of single unit responses in the auditory system of normal and kanamycin treated gerbils can—like those of Fig 10—be constructed using a linear superimposition of two components of nearly opposite phase To show this, Zwislocki assumes that his single unit recordings contain similar responses from inner and outer hair cell groups and that the phase of the response of the outer hair cells has been shifted 180° with respect to the phase of the inner hair cell response With the idea of phase opposition, Zwislocki comments as to a possible mechanism to account for neural sharpening and two tone effects among others

The most substantial evidence for the support of phase opposition has been obtained from kanamycin treated animals Zwislocki reasoned that as it was highly unlikely there should be different stimulations of hair cells along the organ of Corti, one must interpret the single unit recordings with consideration given to phase opposition of equal components It was noted earlier in this paper that the mechanical functioning of the organ of Corti could be altered by removal of the outer hair cell cilia For example, it could be imagined that removal of these cilia would cause the tectorial membrane to collapse on to the reticular membrane, thereby blocking the reticular lamina to fluid motion Thus different sections of the organ of Corti could very well produce substantially different stimulations of the hair cells Furthermore, if the responses of the two receptor groups do interact to sharpen the mechanical stimulation it must be concluded that the response of an inner hair cells should not be as finely tuned as that of the single unit The results of Russel & Sellick (1977) indicate that inner hair cells exhibit the same sharp tuning as the single unit Thus it would appear that the idea of phase opposition of inner and outer hair cell responses is not of revelance to the mechanism of tuning Perhaps the importance of phase opposition lies elsewhere

Earlier in this work, the phase difference between the stimulations presented to the hair cell groups was observed to correspond to an apical shift of the inner hair cell stimulation with respect to the outer hair cell stimulation Thus a given frequency of arch motion f_1 will produce two maximum stimulations at two different locations along the basilar membrane Furthermore, the distance between these maxima is uniquely defined by f_1

It is interesting to speculate on the functioning of the model if now a second frequency of arch motion f_2 is introduced simultaneously with f_1, where the two frequencies differ by a small amount. Following the previous analysis there will exist in the model two maxima of inner and outer hair cell stimulation corresponding to f_1 and a different set of maxima produced by f_2. Because the arch motions are introduced simultaneously, and the frequencies differ by a small amount, the maxima of the two inner hair stimulations will lie in close proximity to each other, as will the maxima of the outer hair cell stimulations. Therefore, the following four distances are defined by the four maxima: between the maximum inner hair cell stimulation of f_1 and the maximum outer hair cell stimulation of f_2; between the maximum inner hair stimulation of f_2 and the maximum outer hair cell stimulation of f_1, between the maximum inner hair cell stimulation of f_1 and the maximum outer hair cell stimulation of f_1; and lastly between the maximum inner hair cell stimulation of f_2 and the maximum outer hair cell stimulation of f_2. If a given frequency produces a unique separation of maxima, then a known separation of maxima would imply a unique frequency. The distance between the maxima of inner and outer hair cell stimulation of f_1 is not equal to that distance corresponding to f_2. Thus the distances defined by the misalignment of the maximum stimulations, the stimulation intermodulation components, should produce frequencies unequal to f_1 or f_2. Approximate calculations for these components show these new frequencies are within 8% of the values $2f_1-f_2$ and $2f_2-f_1$.

While this result is only a speculative consequence from the model, the following point must be emphasized: the model presented herein contains a mechanism which could be considered to produce stimulation intermodulation components without requiring non linear behavior of the basilar membrane. This is a comforting result in light of the data we have on basilar membrane motion.

(f) Vulnerability of the model to chemical influences

As was mentioned earlier, a change in the chemical composition of the endolymph is known to physically alter the tectorial membrane. However, it remains to be shown that the chemical composition of the endolymph is dependent on metabolic processes. Bosher & Warren (1968) investigated the electrochemistry of rat endolymph during anoxia. In the normal state they found the endolymph to have a resting potential of +92 mV with concentrations of sodium and potassium of 0.91 and 154 mEq/liter. During anoxia the positive resting potential became negative, reaching a minimum value of −42 mV after 4½ minutes and thereafter increasing to zero. After 2 min of anoxia, the concentration of sodium attained a value of 3.6 mEq/liter and increased to 32 mEq/liter after 30 min. Correspondingly, there was a decrease in the potassium concentration to 116 mEq/liter. From these results it was concluded that the composition of the endolymph is related to the active transfer of sodium out of and potassium into the scala media, probably via the stria vascularis. But most significantly, these results show the strong dependence of the transfer mechanisms on oxidative metabolism. Thus if the physical properties of the tectorial membrane are a function of the chemical composition of the endolymph, and the chemical composition of the endolymph can be drastically altered by changes in the metabolic state, then the physical properties of the tectorial membrane must be directly related to metabolic processes.

It has been shown by Evans (1975) that both anoxia and the addition of contaminated perilymph to the endolymph causes the recorded tuning curves to change from normal low-threshold sharply tuned curves to high threshold broadly tuned curves. The change in threshold is approximately 30 dB. Both the loss of sharpening and the raising of threshold by 30 dB were predicted by the model for the case where the contracted tectorial membrane

was in physical contact with the inner hair cell cilia

Though this mechanism offers a possible explanation of the effect of anoxia on the organ of Corti, it may be argued that the change in metabolism would primarily affect the nervous system If this were so, we would expect to see an attenuation of the tuning curve that is more or less constant over the range of frequencies examined However, [illegible]ns (1975) has shown, the region of sharp tuning is attenuated to a much greater extent than is the remainder of the tuning curve

In addition to the attenuation, the effects of anoxia on the tuning curve are observed within minutes after the oxygen level is reduced Tonndorf et al (1962) have noted that the changes in the physical properties of the tectorial membrane follow within minutes the change in the composition of the endolymph Thus if the physical properties of the tectorial membrane were to effect the tuning curves in some fashion, they are capable of doing so in the required time However, there is also evidence that the nervous system responds to anoxia within the same short interval of time Spoendlin (1974) has noted a swelling of the afferent dendrites to the inner hair cells within minutes after the start of anoxia It therefore appears that both the nervous system and the tectorial membrane could be affected by anoxia

Whether or not the mechanism proposed by the model functions in the actual organ of Corti can only be proved by experimentation Therefore, as a test of the ideas expressed in this research, it is suggested that an experiment should be undertaken where the changes (if any) in the physical properties of the tectorial membrane could be measured as a function of metabolic state Comparison of the results of such an experiment with the model prediction will be very enlightening

Discussion and Conclusions

Previous models of the organ of Corti have been proposed to explain the increase in sharpening between basilar membrane motion and neural tuning curves (Billone, 1972; Duifhuis, 1976, Allen, 1977) Unfortunately, each of these models is lacking in some degree

An analysis of the mechanics of the organ of Corti has led Billone to conclude that the fluid moving in the reticular lamina could produce the force necessary to stimulate the hair cells However, Billone assumed that the fluid motion in the lamina was initiated by the relative shearing motion between the reticular and tectorial membranes The present analysis has shown that it is the change in cross-sectional area of the spiral sulcus caused by the movement of the arch of Corti that is responsible for the fluid motion in the lamina Therefore, the relative shearing motion resulting from the arch motion is unimportant in the context of inner hair cell stimulation In addition Billone assumed that the fluid moving in the reticular lamina would flow through the cilia bundles located in the lamina As discussed by Kimura (1966) and Lim (1972), there appears to be a substance between the individual cilia of a bundle, probably mucopolysaccharides The effect of this substance is to render the cilia bundle impervious to fluid flow Therefore, fluid moving in the reticular lamina would flow around the cilia bundle rather than through it, as postulated by Billone

The model proposed by Duifhuis utilizes the different types of shearing between the reticular and tectorial membranes as experimentally observed by Bekesy (1960) However, it ap

pears that this model is not capable of generating the required neural sharpening over the necessary range of frequencies (Duifhuis, 1976*a*)

Rather than starting from a physical analysis as the two previous models have done, Allen first describes a non linear mathematical model involving an arbitrary parameter, the loss factor' After an appropriate choice of this parameter and solution of the equations, Allen seeks a physical realization of his mathematical ideas In doing so, he assumes that the inner hair cell cilia are embedded in the tectorial membrane as deeply as those of the outer hair cell cilia This assumption is highly questionable in view of the results of Kimura (1966) Lim (1972) and several others

The model of the organ of Corti presented in this paper is based on a linear mechanical conceptualization of the functioning of the organ of Corti resulting from light and electron microscopic studies of the organ There is no utilization of any arbitrary parameter or assumption The only parameter used in the actual calculations that has not been directly measured is the spring constant of the border–inner hair cell area of the spiral sulcus This constant is however determined from the material properties of the organ of Corti itself The model thus presented herein is of physiological importance in explaining the mechanics of the organ

Viewed in terms of the model results, we can assemble the following picture of the cochlear partition The inner hair cells appear to be responsible for the detection of basic information, i e frequency, while the outer hair cells, could be utilized for 'finer' analyses, two tone effects, etc The stimulus presented to the inner hair cells is sharpened by the interaction of the arch of Corti with the fluid contained in the spiral sulcus Stimulations of the outer hair cells follow basilar membrane motion While both hair cell groups have a unique place of maximum stimulation as a function of frequency, the maximum stimulation of the inner hair cells is located more apically than that of the outer hair cells In addition, loss of outer hair cell cilia or damage to the tectorial membrane results in a marked change or elimination of sharpening

The fact that the model produces a realistic time constant, a sufficient sharpening of neural stimulation predicts an experimentally verifiable single fiber response and several other experimentally observable phenomena as well as being chemically and metabolically vulnerable is very encouraging However, true verification of the hypothesis that fluid motion in the organ of Corti is responsible for the observable neural sharpening can only be obtained from a direct quantitative measurement of this fluid motion

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SUPPLEMENT 364

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Value and significance of « latency » and « amplitude » in absolute sense and in relation to the auditory threshold

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INTRODUCTION

Within the wider field of Electrical Response Audiometry (ERA), the technique known as *Brainstem Electric Response Audiometry* (*BERA*) has been found particularly useful in recent years The results it has supplied so far suggest that its clinical and scientific applications will be further extended

The interest and soundness of BERA as a technique stem from its simplicity and its brief test procedure Moreover, by comparison with methods, such as EEG audiometry, based on the detection of "slow" potentials, it offers decidedly more constant responses that are not affected by changes due to the use of sedatives or dependent on the fact that the subject is awake or asleep

In comparison with electrocochleography (ECochG), BERA has the advantage of not requiring any form of surgery It can thus be performed by non medical staff, and virtually at the out patient level

The criterion employed for differentiation of the electrical responses to acoustic stimulation now rests on numerous data over years of experimentation on the part of skilled laboratories It is primarily based on the "latency" between stimulus presentation and the recording of the response By contrast, no practical value in the absolute sense, has been reported for the voltage, i e the amplitude of the response, especially since the relevant data vary considerably even in the normal subject (Cazals et al, 1978)

Whereas the variations in conduction time, in fact, appear to be devoid of significance, the electrical potentials, which vary in amplitude in relation to the number of fibres activated and are recorded at a considerable distance from their point of origin, determine the amplitude of the response to a large number of variables

It has at all events, been shown that for all the acoustic electrical potentials, from the "immediate" to the "late", there is a direct proportional relation between the intensity of the stimulus and the amplitude of the response Latency, on the other hand, diminishes as the intensity of the stimulus increases

According to the arrangement proposed by Picton et al (1974) and Davis (1976), the auditory electrical potentials can be classified as follows

TYPE	CLASS	LATENCY (msec)		
ECochG	Immediate	1-2	SP	= summating potential
			CM	= cochlear micro-phonics
			AP	= action potential of the cochlear nerve
Vertex (V) potentials	fast	2 8		
	medium	8 50		
	slow	50-300		
	late	300-600		

There is general agreement that the immediate (ECochG) responses originate in the organ of Corti (SP and CM) and the acoustic nerve (AP) The fast potentials are derived from the brainstem, while the medium potentials originate in the thalamus and the first cortical projections (this class also includes myogenic responses) The slow potentials are thought to originate in the cerebral cortex, whereas the late responses constitute what is known as the expectancy wave or Contingent Negative Variation (CNV), this, too, is of cortical origin, but of negligible clinical interest

A further distinction must be made with regard to the position of the recording electrodes ECochG potentials, in fact, are recorded relatively close to their anatomical point of origin, whereas all other potentials are recorded at the vertex, and hence referred to as "V potentials", at various distances from their origin

In the case of ECochG, what is more, the position of the active electrode is critical If it is placed more than a centimetre from the round window, there will be a 9/10 reduction in response amplitude Its position is less important for the "V potentials", especially *Brainstem Electrical Responses (BER)*

Recording of a differential potential, in any case, will always be required between the auricular lobe and the promontory in ECochG, and between the vertex and the mastoid process or earlobe in the case of V potentials

Interest in the fast potentials was first aroused by Sohmer & Feinmesser (1967) These Authors recorded the 8th nerve action potential (AP) from the earlobe A response of 0 5 μV was evoked by a click of 115 dB SPL

Experiments on the cat conducted by Jewett (1970) showed that the early neurogenic responses thus obtained were the result of potentials generated near the several levels of the auditory pathway

Comparative studies in man and animals by Jewett and Williston (1971) indicated that the first of these potentials (N1) is generated at the cochlear nerve Lev & Sohmer (1972) used superficial and intracranial electrodes to obtain simultaneous recordings in the cat They reached the conclusion that the wave sequence was produced by the cochlear nerve (N1), the cochlear nucleus (N2), the superior olivary complex (N3), and the inferior colliculus (N4, N5)

Thornton (1976) has proposed the following anatomical correlation for the series of neurogenic responses typical of the brainstem·

Wave			
Wave	*I*	*Acoustic nerve*	*(N1)*
»	II	Cochlear nucleus	(N2)
»	III	Superior olivary complex	(N3)
»	IV	Lateral lemniscus	(N4)
»	V	Inferior colliculus	(N5)
»	*VI*	*Medial geniculate body*	*(N6)*
»	VII	Thalamocortical auditory radiations	(N7)

This classification is now widely accepted It should be noted that waves VI and VII are poorly significant Indeed, their appearance is very irregular, even in rapidly repeated examinations in the same subject, while their anatomical correlation is very uncertain

All the events so far described occur in the first 8 msec following presentation of the stimulus The data reported by a variety of Authors shown that the waveforms are reasonably constant, especially those of waves I, II and III Such differences as are observed are usually linked to the modality of the examination and related to the bandwidth of the amplifier

Account must also be taken of the possibility of responses from both the ipsilateral and contralateral central auditory pathways In discussing this possibility, Terkildsen et al (1973) have shown the variation of the response to unilateral stimulus, depending on whether it is ipsi- or contralaterally recorded Various reasons have been put forward for the origin of contralateral responses They are most likely the result of a neurological crossover In other words, their primary generators are the nuclei lying along the path of the nerve fibres after the crossover, whereby brainstem activity is led to contralateral sites

Recording of the contralateral responses by transcranial transmission would not seen feasible (Thornton, 1975)

PURPOSE OF THE RESEARCH

Parameters indicative of the normal position have been proposed by all Authors in the field in view of the future application of BER in both a clinical and a scientific setting The results obtained in different laboratories have been more or less uniform Even so, it has been thought essential to establish a standard of values strictly related to the apparatus currently available, so as to keep the variables presented by an examination that is by its very nature, hard to fit into a schematic pattern within as narrow a qualitative and quantitative compass as possible when drawing comparisons with different pathologies

Our prime purpose, therefore, was to establish the parameters for the normal latency and amplitude of the individual BER waves, and to obtain fuller information on the function of the central auditory pathways in relation to their complicated anatomical and physiological constitution

Furthermore it was felt that a search for normal parameters suitable for clinical employment must necessarily be extended to an examination of the responses obtained in subjects with an increased threshold for certain frequencies (especially 3 and 4 kHz), since BERA can and must lend itself to diagnostic and prognostic application in pathologies with a threshold increase due to causes other than those whose existence is sought

In substance, therefore, we have set out to determine whether there is a relation between BER and the audiometric threshold and of what type it is The method used to tackle this question was different from that which extrapolates input/output and input/latency values (i e the ratio between stimulus intensity and response latency and amplitude) (Antonelli, et al 1977)

MATERIAL AND TECHNIQUE

Subjects lay on a couch in a silent Faraday cage with their heads slightly flexed, and were asked to relax as much as possible Needle electrodes were used to keep the resistance as constant as possible, since it was necessary to prolong the examination for a considerable period in some cases The earth electrode was placed on the forehead, the active electrode on the mastoid, and the reference electrode on the vertex

The amplification, averaging and trigger apparatus was a Medelec Amplaid Mk III The bandwidth was set at 32 to 3200 Hz The gain setting was 10 μV and the analysis time 10 msec

The stimulus consisted of an unfiltered 100 μsec click centred on the 2000 Hz frequency It was presented via a shielded TDH 39 earphone at an intensity of 90 db SPL at a repetition rate of 10 per second and with direct polarity The contralateral ear was masked with white noise fed through an insert in the external auditory meatus The off line response was averaged from 1024 clicks

An intensity of 90 dB SPL was chosen because this has proved the most suitable for the various clinical conditions examined At lower intensities, the sequence of peaks and their morphology were usually less regular, though the IV-V complex was fully evident even when the stimulus was near the intensity threshold

Simultaneous recordings of the ipsi- and contralateral responses were studied with the addition of a second vertex mastoid lead, with its own amplification channel and memory

Averaging of the 1024 stimuli thus gave BER in separate traces for the two sides The remaining parameters and modalities were the same as those previously described The traces were displayed on a Tektronix 5103 N oscillograph and photographed with a Polaroid camera

RESULTS

The first part of the study was conducted on 20 subjects with normal hearing aged 18-30 yr Fig 1 shows the features of the BER observed in the test (a) and non test (b) ear of these subjects The sequence of positive and negative peaks is clearly visible in both traces Their waveform is similar to that described by Jewett & Williston (1970), Picton et al (1974), Colletti & Sittoni (1978), and Gibson (1978)

The test ear trace (fig 1a) contains 7 peaks with latencies of 1 6 to 8 2 msec The first peaks display a relatively uniform amplitud

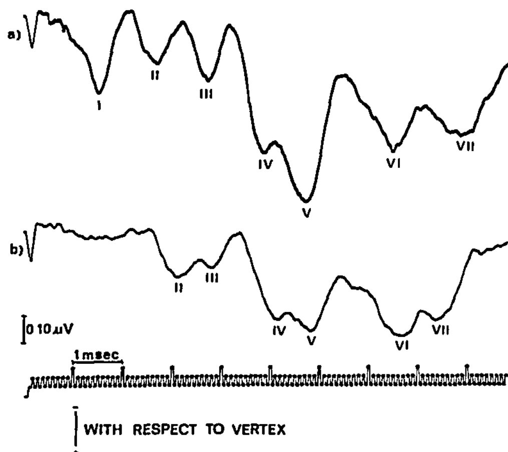


Fig 1 Ipsilateral (a) and contralateral (b) BER to a monoaural stimulus (clicks 90 dB SPL) in subjects with normal hearing Wave IV has the form of a notch in the descending segment of wave V ("*IV-V wave complex*")

Wave IV is similar in shape to a notch in the descending segment of wave V, which is of greater amplitude than the preceding waves Waves IV and V form the "*IV-V wave complex*" They are followed by the last two waves (VI & VII) whose amplitude and morphology are very variable

Wave I is missing from the other trace (fig 1b) Since it is generated at the level of the Corti's organ and the cochlear branch of the eighth nerve, this absence is obvious, since it is the other ear that is stimulated

The subsequent peaks are present and arranged in much the same way as in the ipsilateral response, except that their amplitude is more variable and some waves particularly II & III, may be less evident

Fig 2 illustrates a second, less common type

Table 1 *Normal latency and amplitude values for each BER wave in ipsilateral and contralateral recordings Tested ears 40*

Latency msec	Amplitude µV	Wave	Latency msec	Amplitude µV
1 61 ± 0 08	0 19 ± 0 08	I	—	—
2 82 ± 0 18	0 20 ± 0 09	II	2 79 ± 0 15	—
3 81 ± 0 18	0 25 ± 0 10	III	3 61 ± 0 09	—
5 71 ± 0 21	0 54 ± 0 19	(IV V)	5 68 ± 0 18	0 54 ± 0 12
Ipsilateral		Recording	Contralateral	

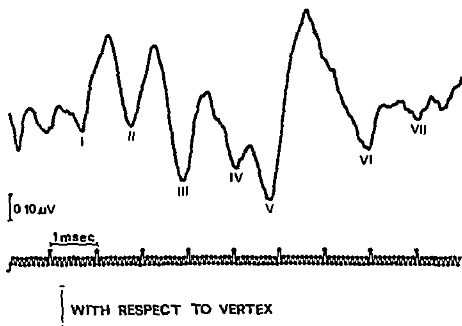


Fig 2 Ipsilateral BER to a monoaural stimulus (clicks 90 dB SPL) in the normal subject Wave IV and wave V are distinct from each other This type of trace is less common than that in fig. 1

Fig 3 Mean value (amplitude/latency) and extension to fiducial limits (2 SD) of ipsilateral BER waves

of trace found in some subjects, using the same amplifier bandwidth Here wave IV is distinct from wave V and the succession of BER peaks is more regular.

To establish the normal parameters for both scientific and possible clinical exploitation of the method, we calculated the latency of the first five waves on the basis of their positive peak, and their respective amplitudes, from the negative peak to the next positive peak, in accordance with the technique adopted by all Authors (Table 1) Waves VI & VII were ignored, owing to their excessive variability

Mean values were plotted with the amplitude (in μV) on the Y axis and the latency (in msec) on the X axis The root mean square deviation and its extension to fiducial limits of 95% (2 SD) were worked out for the individual wave values

The extrapolated data give an ellipse plot for each wave (fig 3) The fourth ellipse refers to the IV-V complex Since wave IV varies considerably in shape, it was treated as part of the complex, as suggested by Gibson (1978) (Table 1) This evaluation enables the data observed in each examination to be assessed Values falling within the ellipse can be regarded as normal, those falling outside as pathological

The method can only be applied to the ipsilateral responses, however, at least as far as our current data are concerned In the case of contralateral recordings wave amplitude varies too widely to enable reliable data to be obtained (Table 1)

The wave IV-V complex provides the only exception Its contralateral graph is illustrated in fig 4 The ellipse represents the fiducial limits of 95% (2 SD) for this complex only (Table 1)

Our findings indicate that latency is the most constant and reliable parameter for both ipsilateral and contralateral potentials The greater variability of the parameter amplitude, other than to obvious bioelectrical causes (neurogenic potentials that are difficult to check, owing to the distance between the point of origin and the point of recording, resulting in low voltages) is very likely also due to the difficulty of pinpointing the measurement from

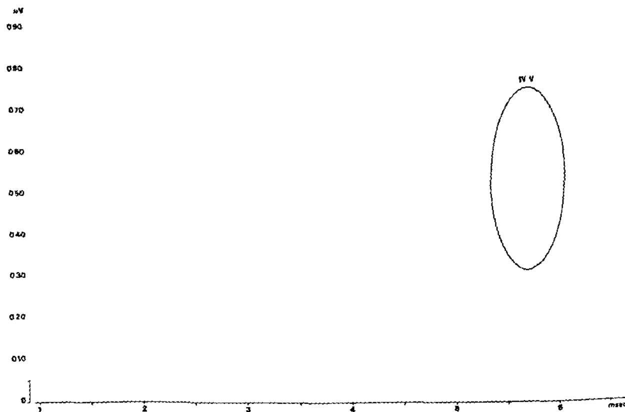


Fig 4 Mean value (amplitude/latency) and extension to fiducial limits (2 SD) of the contralateral IV V complex

a positive peak to the following negative peak on account of the uncertain morphology of the trace

It is clear, therefore, that with the techniques currently available greater reliance must be placed on the latency of the peaks, rather than their amplitude, as the parameter for judging whether or not a BER is normal (Cazals et al, 1978)

Table 1 contains the latency values (± 2 SD) for ipsilateral waves I, II, III and the "IV-V complex", and contralateral waves II & III and the IV-V complex No marked differences between the two sides are apparent In particular, the interaural latency difference (ILD) of 0 03 msec for the IV-V complex is fully in agreement with the < 0 2 msec regarded as normal (Clemis & Mitchell, 1978)

The importance of analysing both ipsilateral and contralateral responses lies in the fact that brainstem pathologies may furnish asymmetrical responses detectable on bilateral comparison (Thornton & Hawkes, 1976)

Table 2 shows the latencies between the wave peaks on both sides The sequential relations,

Table 2 *Normal intra wave latencies (msec) in ipsilateral and contralateral BERA Tested ears 40*

Wave	Recording	
	Ipsilateral	Contralateral
I II	1 21 ± 0 10	—
II III	0 99 ± 0 18	0 82 ± 0 25
III-(IV V)	1 90 ± 0 18	2 07 ± 0 18

which are about one potential per msec, may be altered in certain diseases, and it is this that makes the data useful

Lastly, Table 3 sets out the data for the latency between the "IV-V complex" and the preceding waves Once again, it can be seen that substantially similar recordings are obtained on both sides The latency of about 4 msec between the IV-V complex and wave I agrees with the findings of Terkildsen (1973), Thornton (1976), Gibson (1978), and Paludetti et al (1978)

Table 3 *Normal latencies between the "IV V complex" and the preceding waves in ipsilateral and contralateral BERA Tested ears 40*

Wave	Recording	
	Ipsilateral	Contralateral
I-(IV V)	4 10 ± 0 27	—
II-(IV V)	2 89 ± 0 18	2 89 ± 0 29
III-(IV V)	1 90 ± 0 18	2 07 ± 0 18

The second part of the research was directed to an examination of the behaviour of BER in relation to the air conduction auditory threshold Forty subjects with cochlear hearing losses with recruitment, increased air conduction threshold for frequencies of 3 and 4 kHz up to 80 dB HTL, and hence an extensively various tonal threshold curve pattern were studied A further 40 subjects with occupational hearing losses with recruitment and a threshold increase confined to 3 and 4 kHz were also examined

These two frequencies were chosen because cases in which hearing loss due to chronic acoustic trauma results in a defect confined in this way are commonly encountered

Four "bands" in which the threshold for frequencies of 3 and 4 kHz were between 0-20, 20-40, 40 60, and 60-80 dB HTL were examined for each group Each band was represented by 10 subjects The amplitude and

Table 4 *Subjects with threshold increases not solely confined to frequencies of 3 and 4 kHz Latency and amplitude values of the "IV-V complex" for ipsilateral recordings in relation to the threshold increase for these two frequencies*

Threshold increase dB HTL	Tested ears	Latency msec	Amplitude µV
0-20	20	5 69 ± 0 21	0 55 ± 0 13
20-40	18	5 76 ± 0 21	0 40 ± 0 13
40-60	19	5 97 ± 0 34	0 36 ± 0 11
60-80	16	6 14 ± 0 23	0 30 ± 0 16

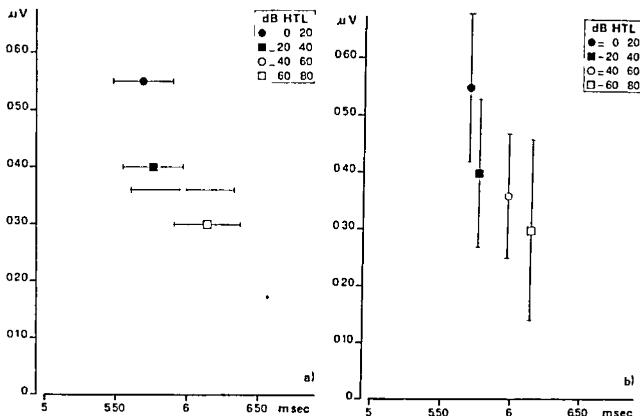


Fig 5 Subjects with threshold increases not solely confined to frequencies of 3 and 4 kHz between 0-20, 20-40 40-60 and 60-80 dB HTL a) mean latency pattern and b) mean amplitude pattern of the " IV-V complex " for each threshold increase, and extension to the fiducial limits

latency (mean and SD) of the ipsilateral IV-V complex were evaluated for each band Attention was restricted to this complex on account of its constant morphology, irrespective of the subject's threshold level

The data for the first group are shown in fig 5 and Table 4 These subjects presented various threshold levels for the other frequencies of the tonal field, in addition to their increased threshold for 3 and 4 kHz Fig 5a shows the latency values There is an inconstantly proportional relation between the threshold and latency increases The increase in latency is less rapid for threshold values up to 40 dB and faster beyond this level Fig 5b makes it clear that amplitude decreases rapidly for threshold increases up to 40 dB, followed by a slower, progressive fall

The data relating to the second group are illustrated in fig 6 and Table 5 These sub-

Table 5 *Subjects with threshold increases solely confined to frequencies of 3 and 4 kHz Latency and amplitude values of the " IV-V complex " for ipsilateral recordings in relation to the threshold increase*

Threshold increase dB HTL	Tested ears	Latency msec	Amplitude µV
0 20	20	5 62 ± 0 17	0 57 ± 0 11
20-40	20	5 71 ± 0 22	0 45 ± 0 15
40 60	18	5 80 ± 0 27	0 41 ± 0 13
60-80	15	6 04 ± 0 25	0 36 ± 0 16

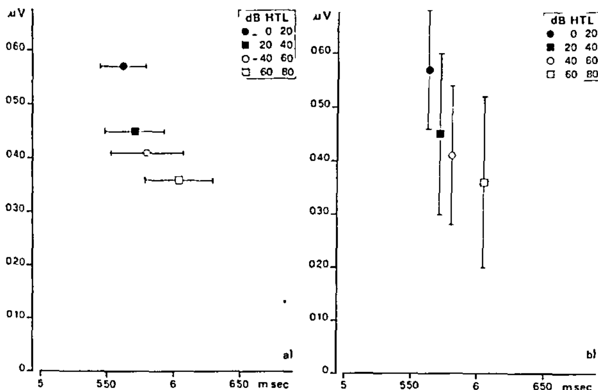


Fig 6 Subjects with threshold increases solely confined to frequencies of 3 and 4 kHz between 0-20 20-40 40-60 and 60-80 dB HTL a) Mean latency pattern and b) mean amplitude pattern of the "IV V complex" for each threshold increase and extension to the fiducial limits

jects presented varying threshold increases for frequencies of 3 and 4 kHz only Both latency (fig 6a) and amplitude (fig 6b) mirrored the patterns observed in the first group though the variations were less extensive This is probably due to the fact that since the stimulus consisted of clicks, the better mean threshold status of the second group influenced their responses, so that changes in latency and amplitude were more restricted (compare figs 5 & 6)

The study was necessarily restricted to frequencies of 3 and 4 kHz for reasons that can be readily understood namely the practical impossibility of finding subjects with a threshold increase gradually rising to 80 dB HTL but confined to one or at most two of the heavy and medium frequencies of the tonal field

The convenience of having reference data for different threshold levels available at any rate for these two frequencies would, however, appear to be amply justified by the fact that the changes provoked by a given pathology in the amplitude and more particularly, in the latency of the "IV V wave complex" must be distinguished from those attributable to threshold increases of cochlear origin, or due to transmission hearing losses so that the percentage of false positives can be kept as low as possible

CONCLUSIONS

Our findings corroborate the view that BERA is based on constant, rapid responses and relatively simple execution modalities

In particular, it was noted that

1) BER morphology, when recordings are taken on the same side as the stimulated ear, is particularly constant and its individual components can readily be evaluated with clicks 90 dB SPL

2) The morphology of the contralateral BER is less constant though its components are



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SUPPLEMENT 365

Penetration of Erythromycin Through Respiratory Mucosa

A Study Using Secretory Otitis Media as a Model

BY

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Introduction

Following its introduction 25 years ago erythromycin was assigned a reserve place in the therapeutical armamentarium as a last defence against penicillin resistant staphylococci or as an alternative in cases of penicillin allergy In recent years, however, erythromycin has gained a wider acceptance as a first choice antibiotic Concomitantly, there has been an increased interest in its pharmacokinetic characteristics The penetration of erythromycin has been studied in human semen (Eliasson et al , 1978), in bronchial secretion in chronic bronchitis (Fraschini et al , 1978), in maxillary sinus secretion in chronic sinusitis (Paavolainen et al , 1977) and in acute purulent sinusitis (Axelsson & Brorson, 1974, Kalm et al , 1975) Some aspects of the absorption of ery thromycin were recently investigated by Malmborg (1978)

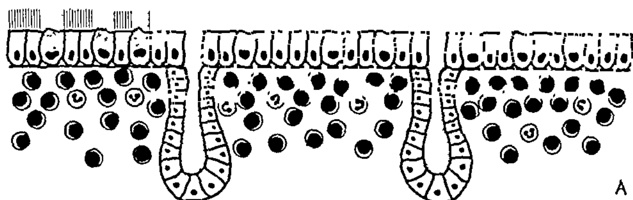
The respiratory mucosa consists normally of ciliated columnar cells with some goblet cells, covered by a thin layer of mucus (Tos & Bak-Pedersen 1976) Morphologically and physiologically the middle ear mucosa constitutes a part of the upper airway system (Flisberg 1966, Sadé, 1966*a*, Henzer, 1970, Lim, 1974, Hagan, 1977) The respiratory mucosa reacts in a uniform manner to an inflammatory stimulus In Secretory Otitis Media (SOM) the response of the mucosa conforms to this general pattern, which is characterized by an increased number of mucus glands and goblet cells, producing an abundance of more or less mucoid secretion (Sade, 1966*b*, Sade & Eliezer, 1970, Bak Pedersen & Tos, 1971, Tos, 1976) This uniform mode of response is also seen in bronchitis and sinusitis SOM may thus be regarded as a suitable model for the study of antibiotic penetration of respiratory mucosa, and the results should be valid for inflammatory diseases in any area lined with respiratory epithelium (Fig 1 A–D)

The aim of the present investigation was to study the penetration of erythromycin through respiratory mucosa, using SOM as a model

Material and Methods

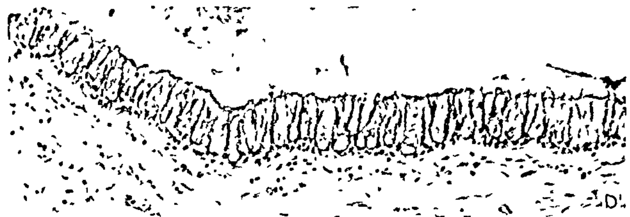
The present study is based on 108 patients with SOM who attended the ENT clinic, Central Hospital, Karlskrona Patients participating in the investigation fulfilled the criteria of SOM fluid behind an intact ear drum at otomicroscopy, and a non purulent middle ear effusion at myringotomy Only patients who did not respond to conventional therapy with decongestants were accepted for the study Cases of middle ear effusions caused by malignant diseases or barotrauma were excluded

Before myringotomy erythromycinethyl succinate (Abboticin®) was administered orally in three doses daily (morning, noon and evening) according to recommended standard dosage (Table I) for different periods of time The patients were divided into nine groups to


A


B


C


D

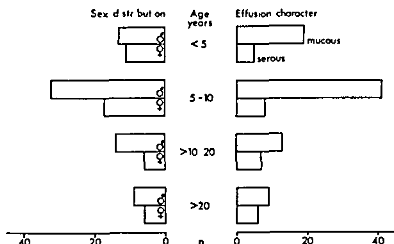


Fig 2 Distribution according to age sex and the character of the effusions

investigate the early and late input phase, the middle ear steady state and the output phase In the input phase myringotomy was performed at 2 and 14 hours (early input, after the 1st and the 2nd dose) and at 26 and 38 hours (late input, after the 4th and the 5th dose) after initiated treatment The middle ear steady state is here represented by sampling after 2 and 10 days of administration The output phase comprises groups where myringotomy was performed 14, 26 and 38 hours after completion of 10 days of treatment

As a rule otomicroscopy and myringotomy was performed under general inhalation anesthesia The median interval between the last dose and myringotomy was 2½ hours except when the output phase was studied The middle ear effusion was aspirated with a sterile suction tip and collected in a sterile plastic tube which was sealed to avoid evaporation Specimens contaminated with blood were discarded Blood samples for determinations of the plasma concentration were drawn simultaneously to myringotomy The middle ear effusions were assessed according to their viscosity as either serous or mucous Fig 2 shows the distribution according to sex, age and the character of the effusions The majority were children with a predominance of boys In the younger age groups (<10 years) the ratio between mucous and serous effusions was 4 6/1, in the older children and adults (>10 years) the ratio was 1 7/1

Fig 1 (A) Schematic drawing of respiratory tract mucosa representative of SOM chronic sinusitis and chronic bronchitis Shaded area indicates mucous blanket (B) Middle ear mucosa in a case of SOM Htx-eosin ×250 (C) Maxillary sinus mucosa in chronic sinusitus MacManus ×650 (D) Bronchial mucosa in chronic bronchitis MacManus ×210

Assay of erythromycin in plasma and middle ear secretions

An agar well diffusion method on DST agar (Oxoid), pH 7 6, was used Plates (23×23 cm) were inoculated with *Sarcina lutea* ATCC 9341 by flooding Wells containing about 20 μl (diameter 2 6 mm) were punched out and filled to the brim with samples The plates were incubated at +37°C overnight Two diameters at right angles to each other were measured around each diffusion centre, the mean being taken as the diameter of the inhibition zone

Table I *Erythromycin dosage scheme*

Weight kg	Daily doses g	Dose/day g	Dose/weight mg/kg
10–14	0 2+0 2+0 2	0 6	60–42
15–19	0 2+0 2+0 4	0 8	53–42
20–25	0 4+0 4+0 4	1 2	60–48
26–90	0 4+0 6+0 6	1 6	62–18

Table II *Comparison between regression lines obtained by erythromycin concentrations 4 0–0 13 mg/l in plasma versus in middle ear secretions*

	Experiment number										Total
	1	2	3	4	5	6	7	8	9	10	
t_{par}	1 14	0 42	0 39	0 52	0 27	0 16	1 66	0 52	0 29	0 93	
t_{id}	9 85	5 87	6 74	8 03	5 42	7 71	4 07	11 8	24 1	4 51	
Percentage concentration[a]	66±7	64±10	72±8	59±9	74±9	71±7	79±9	70±5	70±6	73±12	70±9

t_{par} t test for parallelity All values give $p>0$ 20
t_{id} t test for identity All values give $p<0$ 001
[a] $\frac{\text{Concentration calculated from erythromycin/plasma regression line}}{\text{Concentration calculated from erythromycin/middle ear secretion regression line}} \times 100$

Each sample was determined in duplicate on the same plate

Plasma stock solution

Erythromycin U S P. base lot no 48-050-CD was obtained from Abbott Laboratories, Chicago, Ill , USA, and had a certified potency of 940 mg/g 851 05 mg of dried substance was dissolved in 100 ml methanol Five ml of this solution was diluted to 50 ml with pooled heparinized human plasma from healthy blood donors and the pH was adjusted to 7 6 This stock solution of erythromycin in plasma containing 800 mg/l was divided into aliquots and stored at −20°C

Standard solutions

Middle ear secretions from children with untreated SOM were collected and pooled The effusions had too high a viscosity for serial diluting and therefore had to be treated with a 20% solution of *N*-acetyl cysteine (Inspir®), until precision pipetting with small volumes could be carried out The amount of *N*-acetyl-cysteine solution required was roughly 10% of the final volume Four standard solutions of erythromycin in the concentration range 4 0–0 13 mg/l were prepared from the plasma stock solution by serial double dilutions using the following diluents pooled middle ear secretions with roughly 2% *N* acetyl cysteine, plasma, plasma with 2% *N* acetyl cysteine, and 20% *N* acetyl cysteine

Regression lines

To determine regression lines ten experiments were carried out, each consisting of testing the four standard solutions in duplicate on the same plate The regression lines were parallel in each experiment (0 10<p<0 90) The inhibition zones produced by erythromycin in pooled middle ear secretions were significantly smaller (p<0 001) than those produced by the antibiotic in the other three diluents Between these latter no significant difference was found (0 05<p<0 90) The concentrations of erythromycin in pooled middle ear secretions, as calculated from the regression line produced by the standard solutions in plasma were thus found to be lower than the expected values The range was 59–79%, mean 70% with a S D of 9% (Table II) The

Table III *Erythromycin concentrations in human plasma*

Control solutions See text

	Solution		
	I	II	III
Calculated concentration	0 4	1 6	3 2
Number of determinations	61	64	46
Mean	0 43	1 63	3 28
Range	0 33–0 56 (77–130)	1 34–2 00 (82–123)	2 40–3 92 (73–120)
S D	0 05 (12)	0 14 (9)	0 34 (10)

Figures within parentheses denote concentrations expressed as % of mean

Table IV *Erythromycin concentration in middle ear effusions*

Repeated determinations

	Sample								
	1	2	3	4	5	6	7	8	Total
n	6	6	8	8	6	5	6	5	50
Mean	0 29	0 49	0 60	1 14	1 12	1 70	1 44	2 32	(100)
Range	0 16–0 35	0 29–0 65	0 47–0 65	0 95–1 41	0 91–1 33	1 30–2 17	1 22–1 80	1 78–2 77	
	(55–121)	(59–132)	(78–125)	(83–123)	(82–119)	(76–126)	(85–125)	(77–119)	(55–132)
S D	0 07	0 13	0 10	0 15	0 14	0 36	0 21	0 48	
	(24)	(26)	(16)	(14)	(13)	(21)	(14)	(21)	(18)

Figures within parentheses denote concentrations expressed as % of mean

experiments were performed in 3 days during which time the activity of erythromycin in the *N*-acetyl cysteine containing solutions did not decrease

Assay routine

Aspirated middle ear secretions and heparinized capillary blood arrived at the laboratory one day after collecting Secretions and plasma were assayed immediately against standard solutions of erythromycin in plasma The concentrations of erythromycin in middle ear secretions were calculated as 1 4 times the concentrations obtained from the regression line produced by the plasma standard solutions (cf Table II)

Plasma controls

Erythromycin in plasma in concentrations of 0 4, 1 6 and 3 2 mg/l was prepared from the plasma stock solution, divided into aliquots and stored at −20°C At least one of these controls was tested on every assay plate The results are shown in Table III

Multiple determinations of erythromycin concentration in middle ear secretions

On eight occasions the supply of middle ear effusion was sufficient for redetermining the concentration 5–8 times These samples were stored at +4°C and redetermined once or twice daily during the following days until all the material was used None of these samples were stored for more than 5 days Table IV shows the results

Non-specific antibacterial activity

Whenever possible what was left from the sample of middle ear secretion was added to a well in DST agar and inoculated with an erythromycin resistant strain of *Sarcina lutea* isolated locally Non specific antibacterial activity was not observed

Results

The results are presented in Fig 3 and Table V

Erythromycin in middle ear effusion

In the early input phase there was a slow penetration of erythromycin into the middle ear effusion Two hours after the initial dose the mean value was <0 13 mg/l However, 12 hours later, i e about 2 hours after the second dose, the mean level was 0 6 mg/l Not until in the late input phase, after 26 and 38 hours, did the concentration of the middle ear effu

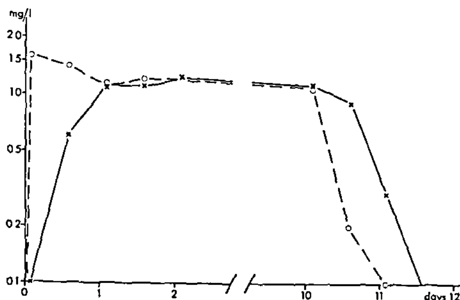


Fig 3 Erythromycin concentration in middle ear effusion and in plasma — concentration in middle ear effusion concentration in plasma All values shown are the means of 10 or more determinations except on day 11½ where there are only two cases Shaded area denotes erythromycin therapy

sion reach its plateau level of about 1 1 mg/l This level was maintained during the middle ear steady state, being 1 2 mg/l on day 2 and 1 1 mg/l on day 10

The output phase was characterized by a slow elimination 14 hours after the last dose of 10 days' treatment, the mean middle ear effusion level was still as high as 0 9 mg/l, implying a maintained middle ear steady state 26 hours after the last dose, however, the elimination had proceeded and the erythromycin concentration had decreased to 0 3 mg/l Another 12 hours later there was no erythromycin activity left in the effusion This implies a biological half life of about 12 hours in the secretions, should the elimination proceed rectilinearly

Erythromycin in plasma

Two hours after the initial dose the mean plasma level was 1 6 mg/l and 12 hours later, i e 2 hours after the second dose, it was 1 4 mg/l The mean plasma levels were then maintained at 1 1–1 2 mg/l up to the 10th day In the output phase there was a rapid elimination 14

Table V *A survey of the results of the entire series*

	Early input		Late input		Middle ear steady state		Output phase		
	2 h	12 h	24 h	36 h	48 h	10 d	12 h	24 h	36 h
Plasma									
Mean mg/l	1 6	1 4	1 1	1 2	1 2	1 1	0 2	0 1	0 0
S D	0 69	1 1	0 5	0 6	0 9	0 8	0 2	0 1	
Range	0 5–3 0	0 5–4 2	0 6–2 0	0 2–1 8	0 4–3 2	0 0–4 2	0 6–0 4	0 0–0 3	
n	10	10	10	11	14	27	12	12	2
Ear eff									
Mean mg/l	0 1	0 6	1 1	1 1	1 2	1 1	0 9	0 3	0 0
S D		0 5	0 7	0 5	0 6	0 9	0 7	0 3	
Range	0 0–0 4	0 3–1 5	0 3 2 2	0 2–2 3	0 4–2 7	0 4–4 2	0 0–2 5	0 0–0 8	
n	10	11	13	16	17	27	12	17	2
Cultures									
n	8	10	10	7	8	27	12	8	2

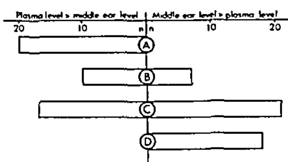


Fig 4 A graphical demonstration of the course of penetration (A) Early input phase (B) late input phase (C) middle ear steady state and (D) output phase Cases with identical concentration in plasma and middle ear effusion are not included

hours after the last dose of 10 days' treatment the mean plasma level was down to 0 16 mg/l, and a further 12 hours later it was <0 13 mg/l It is generally known that the plasma half life of erythromycin is in the order of 2 hours (e g Malmborg, 1978)—in sharp contrast to the estimated half life in the middle ear effusions, which was in the magnitude of 12 hours

General aspects

Fig 4 displays graphically the course of the penetration In the early input phase the individual determinations showed a higher concentration in plasma than in the effusion In the late input phase and at the middle ear steady state there was a balance in this respect The output phase was a mirror image of the input phase here the middle ear effusions contained more erythromycin than plasma

According to recommended standard erythromycin dosage, children are given 40–60 mg/kg body weight/day whereas adults receive less Table VI demonstrates the results of

Table VI *The age distribution of the erythromycin concentration in plasma 2 hours after the last dose (n=82) and in middle ear effusion on the 2nd and 10th day (n=41)*

Observe that despite different dosages (mg/kg) the mean levels are similar

	Age group years			
	<5	5–10	>10–20	>20
Plasma				
M mg/l	1 3	1 2	1 0	1 3
S D	0 9	0 8	0 7	1 1
n	22	33	16	11
Ear eff				
M mg/l	1 1	1 0	1 4	1 1
S D	0.5	0 8	1 3	
n	11	18	9	3

plasma concentration and effusion levels at middle ear steady state in different age groups There was no significant difference despite the dissimilarity in dosage

As there was only a small number of serous secretions the material does not allow a comparison of the penetration of erythromycin into secretions of different viscosities However, the values obtained in serous secretions were in good agreement with those in mucous

It has been stated that middle ear secretions (Sirala & Lahikainen, 1952), like sinusitis secretions (Kalm et al , 1975) have an antibacterial effect of their own Such an antibacterial effect could not be demonstrated in our material

In 17 cases samples were assessed from both ears The results tallied very well—identical in 9 cases and in 7 cases there was a difference of less than 20% between the pair of ears In only one case was there a marked dissimilarity

Discussion

It should be pointed out already at the beginning that the present investigation was not undertaken to propose antibiotic treatment of Secretory Otitis Media (SOM)—conventional treatment including the use of grommets is still an unsurpassed mode of therapy However

SOM is in our opinion a very suitable model for studying antibiotic penetration of respiratory mucosa (Sundberg et al., 1978).

In recent years interest in the penetration of antibiotics has increased considerably. There has been a lack of knowledge of the penetration characteristics, even as regards antibiotics that have been in use for several decades, such as erythromycin. The reason for this may be the earlier view that the plasma concentration was the only salient parameter. It has become increasingly evident that the concentration of an antibiotic in plasma does not give sufficient information about its ability to penetrate into the tissues. Taken to the extreme, it is not unlikely that a very high plasma concentration of an antibiotic implies that it is confined to the blood and therefore penetrates the tissues to a smaller extent than may be surmised (Norrby, 1978). In modern pharmacokinetics, attention has shifted to the concentration of the antibiotic in the very focus of inflammation.

Penetration studies may be performed in several different ways. Interstitial fluid concentrations can be determined by inert chamber (e.g. Holm et al., 1978, Norrby et al., 1978, Chisholm, 1978) or skin blister methods (e.g. Simon, 1976, Schreiner et al., 1978). Direct determination of tissue concentrations is another useful technique, although blood contamination is an inherent source of error that may influence the results. The numerous samples needed to gain a general understanding of the penetration process is another obstacle in methods applied directly to tissues, especially in areas such as the middle ear where biopsies cannot be performed indiscriminately. Studies of secretions are commonly performed and offer many advantages. Normal and pathological secretions are often available in sufficient amounts to enable determinations. It is now generally accepted that antibiotic concentration in a secretion is always lower than in its mucosa (Eneroth, 1976, Eneroth & Lundberg, 1976). Adequate concentration in the effusion implies more than adequate levels in the mucous membranes. Albeit determination of antibiotic penetration into secretion from the middle ear is an indirect way, it still affords an estimation of the mucous membrane concentration. Thus studies based on secretion are informative without loss of reliability and relevance.

SOM is often seen as a non-purulent continuation of a purulent otitis media. In other cases the debut is insidious, most often in connection with an upper respiratory infection. In its initial stage there is an increased number of goblet cells and a development of mucus glands in the epithelium (Tos & Bak-Pedersen, 1973*a*, *b*, Sadé, 1974, Tos & Bak-Pedersen, 1977). The second stage is characterized by an accumulation of mucus in the middle ear to such an extent that the clearing action of the mucociliary system is overloaded. This stage has a long duration of several months or more. Thus there is a long period during which sampling can be performed with such a uniform case material that inter-individual differences may be considered very small. Eventually the disease enters the stage when mucus glands degenerate, the epithelium returns to its normal state and resolution takes place.

SOM has been the subject of many studies, most of them focused on therapy and, in recent years, on etiology. The penetration of antibiotics has been investigated in *only a few* instances. The use of SOM as a model for the study of antibiotic penetration on the respiratory mucosa has not been proposed earlier.

Our choice of SOM for penetration studies is based upon the following considerations. The middle ear mucosa is a part of the upper respiratory tract—embryologically, morphologically and physiologically. Its histological picture conforms to the general pattern of respiratory mucosa, with ciliated columnar epithelium intermingled with goblet cells (Bak-Pedersen & Tos, 1973). In inflammatory diseases the middle ear mucosa reacts in the same way as other parts of the upper respiratory tract mucosa, with a considerable increase in the number of goblet cells and development of

mucus glands both producing a large amount of mucus Therefore the results of penetration studies into the middle ear effusion would be valid not only for the middle ear itself but for the whole upper respiratory tract

Earlier reports on the penetration of antibiotics have mainly focused on the input phase, fewer have dealt with later stages of the course, and the output phase is generally disregarded For a comprehensive examination of penetration, all three aspects should be clarified Consequently many samples have to be obtained during the whole period of antibiotic treatment In clinical practice this often presents difficulties as, on one hand, the number of individual cases must be large and, on the other, the material must be as uniform as possible

In cases of SOM which do not respond to conservative therapy, myringotomy with insertion of a grommet is the standard procedure The middle ear effusion does not recur as long as the grommet is functioning Therefore, in using SOM as a model for penetration studies we are confined to a single sample of secretion per case However SOM is a very common condition and the clinical picture is singularly uniform, as is the histological appearance of the mucosa At grommet insertion the middle ear effusion is always aspirated and samples are thus obtained without extra procedures Furthermore, contamination with blood seldom occurs and is easily detected in the otomicroscope Moreover, the samples are taken under aseptic conditions

Ideally, the erythromycin concentration in middle ear effusions should be assayed by means of a set of concentration standards in the same type of secretion The amount of middle ear effusion that could be obtained for standard solutions was not sufficient for the large number of determinations required in this study Furthermore the viscous middle ear secretions had to be pretreated in order to reduce their viscosity and *N*-acetyl-cysteine was used for this purpose Diffusion of erythromycin from these pretreated SOM secretions was slower than from plasma, giving smaller zones of inhibition Evidently this was not due to the presence of *N*-acetyl-cysteine, since the same amounts of this substance in plasma did not influence the activity of erythromycin in our regression line experiments Moreover, the reduction of the zone diameters was of the same magnitude as observed by Kalm et al (1975) when they compared the diffusion from sinus secretions and serum, using essentially the same technique

In our experiments the regression lines from middle ear secretions and plasma were not identical but parallel, permitting us to calculate the erythromycin concentration in the middle ear effusions from the plasma concentration standard lines with a correction factor of 1 4 (Table II) The assay of erythromycin was less accurate for SOM secretion samples (Table IV) than for plasma (Table III), mostly due to difficulty in filling the wells right to the brim with the sometimes very viscous material The accuracy was nevertheless considered adequate for the purpose of this study The good agreement of the results when samples from both ears of the same patient were determined supports this opinion

There are only a few previous studies on antibiotic penetration into the middle ear effusion in cases of SOM Silverstein et al (1966) reported nine cases of SOM where five were given penicillin and four received oxytetracycline i m in a single dose Sampling was performed 1–3 hours later There were only traces of oxytetracycline in the middle ear secretions, penicillin penetrated in four cases but in small amounts Lahikainen (1970) studied the penicillin concentration in 22 cases of SOM, one and 2–4 hours after a single i m dose There were traces of penicillin in the effusions in only nine cases, mostly at 2–4 hours after the administration Lahikainen et al (1977) compared the penetration of azidocillin and ampicillin, given in a single standard dose 74 samples were obtained from 63 patients at 1, 2, 8 and 12 hours after oral administration Both penicillins penetrated

slowly and only low levels were found Klimek et al (1977) studied the concentration of amoxicillin and ampicillin 1–2 hours after a fourfold standard single oral dose in 28 cases of SOM Amoxicillin penetrated four times better than ampicillin Thus earlier studies on antibiotic penetration in cases of SOM have all been performed on smaller series, have merely comprised the early input phase and all of them have been single-dose procedures Erythromycin has not been investigated at all

In the present investigation the penetration of erythromycin through the respiratory mucosa in cases of SOM was followed and elucidated during the whole course—early and late input phase, middle ear steady state and output phase Our results show that erythromycin penetrates into the middle ear secretion, albeit with a slow input phase Highest middle ear values were obtained 24 hours later in the ear than in plasma In the output phase the clearance of erythromycin from the middle ear secretion was also slow The elimination was not completed until more than 24 hours after the last dose given In the middle ear steady state the concentration of erythromycin was the same in middle ear effusions as in concomitantly drawn plasma samples It should be observed that the plasma levels were invariably determined about 2 hours after the last dose given—except in the output phase—and that they therefore represent approximately plasma peak levels Thus the concentration of erythromycin in the middle ear effusion reaches a level which corresponds to the plasma peak level It is a moot question whether erythromycin penetrates merely by diffusion or if there is also a mechanism for active secretion

In the output phase the mean concentration of erythromycin in the middle ear secretion 12 hours after the last dose was still as high as 0 9 mg/l, having only decreased from 1 1 to 0 9 mg/l At the same time the plasma level was as low as 0 16 mg/l—a decrease from 1 1 to 0 16 mg/l These facts show that the middle ear effusion has the ability to retain erythromycin for a considerable time Malmborg (1978) has shown that even after 7 days of oral erythromycin treatment three times daily the plasma levels still vary considerably, the peak level being about five times higher than the lowest value The plasma half life is about 2 hours Therefore, when during interdose intervals the plasma level decreases below the peak level, the erythromycin concentration in the middle ear effusion remains essentially unchanged at the plasma peak value Thus erythromycin not only penetrates the respiratory mucosa in the middle ear to attain levels in the secretion equivalent to plasma peak levels, but it is also retained in the middle ear effusion for a relatively long period of time This fact allows us to use the term 'steady state' for the middle ear secretion

The calculated half-life in the effusion during the output phase was about 12 hours This should be compared with a plasma half life of about 2 hours Now, there are no specialized structures for resorptive purposes in the middle ear, nor is there any evidence supporting the idea that erythromycin might be metabolized in the effusion An antibiotic that is trapped in the middle ear secretion can be eliminated only through two routes by mucociliary clearance via the eustachian tube and by rediffusion into plasma *As erythromycin is retained in the secretion* the output phase apparently reflects a slow mucociliary clearance In SOM there exists a state of equilibrium between the production of secretion and its elimination via the eustachian tube The turnover rate is still unknown (Sade, 1967) According to the discussion above one may expect a turnover rate of not less than 12 hours In this context it would be interesting to study whether there is any difference in the input–output characteristics between mucous and serous secretions, possibly reflecting differences in mucociliary activity The highly viscous mucus ought to impede mucociliary clearance more than the serous secretion (Sadé et al , 1975) However, our series comprises too few cases with serous secretions to

allow us to draw any conclusions on this aspect

A contributing factor to the slow penetration and elimination phases in SOM may be the fact that the antibiotic has to pass into and out from an essentially closed cavity filled with a more or less mucoid secretion Though the mucosa shows all the signs of an inflammatory process there is nevertheless a pronounced difference in degree between the vascular response in SOM and in acute purulent otitis media Thus the general condition in SOM may in itself constitute a partial physiological barrier for antibiotic penetration More far-fetched explanations for a prolonged elimination may be based upon the hypothesis that erythromycin has a special affinity for some constituent in the effusion or has advantageous properties of solubility in it It is still a matter for speculation how other antibiotics would penetrate the respiratory mucosa in SOM The macrolides and the tetracyclines are partially soluble in lipids, whereas the penicillins are not The field is open for further investigations

As a rule bacterial inflammations of the upper respiratory tract display a shorter acute purulent phase superseded by a longer mucus productive stage The inflammatory response of the mucosa in SOM represents this later stage of an acute inflammation In fact, there is overwhelming circumstantial evidence for the inflammatory pathogenesis of SOM—such as the histological appearance of the mucosa (Bernstein & Hayes, 1971, Lim & Birck, 1971, Lundgren & Rundcrantz, 1976, Sade & Weissman, 1977) and the presence of high levels of immunoglobulins and lysozymes in the effusion (Liu et al 1975, Palva et al , 1976, Juhn et al , 1977) Many studies have demonstrated the presence of aerobic bacteria in middle ear effusions in an incidence that varies from 22% to 77% (Senturia et al , 1958, Healy & Teele, 1977, Palva et al , 1978) Bacterial cultures of aural effusions yielded several strains of bacteria, most frequently *Staphylococcus epidermidis* diphtheroid species, *Haemophilus influenzae* and various strains of streptococci

It is well known that the penetration of antibiotics is enhanced during the acute purulent phase However, what happens to the penetration of antibiotics when the inflammation changes to the more protracted mucoid stage? Lundgren & Rundcrantz (1976) followed the penetration of penicillin into the middle ear secretion in acute otitis media during a 6-day course of therapy During the purulent stage, the first day, ample concentrations of penicillin were obtained, being about half the serum level However, later in the course of the disease, when the secretion had turned mucoid in appearance and similar to that in SOM, very much lower levels of penicillin were found in the middle ear effusions, about 10% of the concentration in serum Thus penicillin readily passed into the ear secretion in the acute phase—the first day From the second day on its penetration decreased considerably as the disease turned into a stage similar to SOM There is a similar observation in cases of acute sinusitis Lundberg & Malmborg (1973) found in the initial stage a good penetration of penicillin On the 4th to 5th day, when the secretion had turned mucoid and SOM like, low concentration of penicillin was obtained, less than 20% of plasma peak levels Tetracycline, on the other hand, attained concentrations in the mucoid sinusitis secretion that were equal to and even sometimes surpassed the peak plasma levels

There is a striking parallelism between the penetration of tetracycline in cases of sinusitis and erythromycin in cases of SOM Both antibiotics readily passed into these mucus filled bone enclosed cavities lined with respiratory mucosa, and both antibiotics there attained concentrations equal to plasma peak levels It should be observed that it is during the later, mucoid stage of the inflammation that the scales are tipped—either the mucosa returns to normalcy or it becomes irreversibly damaged, the disease process turning into a chronic condition A suitable antibiotic that penetrates this mucoid stage may well turn the balance in favour of resolution

There are only a few studies on the penetration of erythromycin in other localities of the respiratory tract Axelsson & Brorson (1974) used erythromycin estolate 1 g daily orally for 10 days and found in the secretion of acute maxillary sinusitis a mean concentration of 1 8 mg/l 6 hours after the last dose Kalm et al (1975) studied 20 patients with acute sinusitis half were given 1 5 g erythromycin stearate daily the other half received 1 0 g daily Sinus secretions were obtained on the 3rd and 5th day about 4 hours after the last dose and the mean concentration of erythromycin in the first group was 1 3 mg/l in the second 0 6 mg/l Eleven samples were purulent nine were mucous Paavolainen et al (1977) studied 15 patients with chronic maxillary sinusitis using erythromycin stearate 1 5 g daily orally The average concentration in the secretion after 4 days of treatment was 1 2 mg/l and in the mucosa 1 8 mg/l The last dose was given 2 hours before sampling The penetration of erythromycin stearate into bronchial secretions in cases of chronic bronchitis was determined by Fraschini et al (1978) They found a mean concentration of 1 0 mg/l These reports support our concept that antibiotic penetration of the respiratory mucosa is largely independent of its location

Conclusion

Secretory otitis media has been shown to be an appropriate model for penetration studies the results of which are applicable to the entire upper respiratory tract Erythromycin penetrated into the middle ear effusion and from 26 hours the mean concentration of erythromycin in the effusion was equal to the mean plasma peak level The output phase in the secretion was considerably prolonged compared with the rapid elimination of erythromycin from plasma

Summary

This study is based upon the concept that the respiratory mucosa reacts in a uniform manner to an inflammatory stimulus Secretory Otitis Media (SOM) may be used as a model to disclose some aspects of antibiotic penetration Erythromycin was given for different periods of time to 108 cases of SOM where myringotomy was indicated The middle ear effusion was aspirated and blood samples were obtained simultaneously The concentration of erythromycin was determined by microbiological procedures

Erythromycin penetrated into the middle ear effusion After the fourth dose the concentration was at the same level as the plasma peak level The elimination of the drug from the middle ear secretion was considerably prolonged compared with the rapid elimination from plasma This implies that erythromycin attains a steady state in the middle ear effusion with concentrations equal to the plasma peak level

Zusammenfassung

Die vorliegende Studie basiert auf dem Konzept dass die respiratorische Schleimhaut gleichartig auf einen infektiosen Reiz reagiert und somit die serose Otitis media als Modell

zur Aufschlusselung einiger Aspekte der Anti biotikapenetration dienen kann In 108 Fällen von seroser Otitis media mit Indikation zur Myringotomie wurde Erythromycin in ver schiedenen Zeitintervallen praoperativ verab reicht Die Erythromycinkonzentrationen im Aspirat der Paukenhohle sowie in den simul tan entnommenen Blutproben wurden durch mikrobiologische Methoden ermittelt

Es zeigte sich dass Erythromycin in Paukenerguss bereits nach der vierten Dosis das Niveau der maximalen Plasmakonzentration erreichte Im Vergleich zur schnellen Aus scheidung aus dem Plasma ist die Ausschei dung aus dem Mittelohrsekret bedeutend ver langert Es ist daher anzunehmen dass Erythromycin im Paukenerguss eine steady state Konzentration erreicht die der maxi malen Plasmakonzentration entspricht

Résumé

Cette etude est basee sur la notion que la muqueuse respiratoire réagissant d une manière uniforme a un stimulus inflammatoire on peut se servir de l Otite Secréteuse Mediane (SOM) comme modele pour exposer certains aspects de penetration des antibioti ques Dans 108 cas de SOM ou la myringo tomie etait indiquée la décharge de l oreille médiane fut aspirée et la quantitee d erythromycin fut determinee par procédes microbio logiques Des analyses de sang furent ob tenues par la meme occasion Apres la quatrième dose la pénétration d erythromycin dans la décharge de l oreille mediane atteignit deja des concentrations égales au niveau maximum du plasma

En comparaison l elimination d erythromycin de l oreille mediane est considerable ment plus prolongee que l élimination du plasma Cela implique que l erythromycin at teigne les concentrations stables (*steady state*) de l oreille mediane egales au niveau maxi mum dans le plasma

Resumen

Este estudio se basa en que la mucosa respira toria *reacciona de una manera uniforme a un* estimulo inflamatorio La Otitis Serosa Media (SOM) puede servir de modelo para exponer ciertos aspectos de la penetración de los anti bioticos En 108 casos de SOM en donde la myringotomia estaba indicada la secreción del oido medio fue aspirada y la cantidad de erit romicina se determino por procedimientos microbiologicos Al mismo tiempo analisis de sangre fueron hechos Despues de la cuarta dosis la penetracion de eritromicina en la se *crecion del oido medio* alcanzó ya concentra ciones iguales al nivel maximo en el plasma

En comparacion la eliminacion de eritromi cina del oido medio es cosiderablemente mas prolongada que la eliminacion del plasma Esto significa que la eritromicina alcanza las concentraciones estables (*steady state*) del oido medio iguales al nivel maximo en el plasma

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ADVANCES IN MEASUREMENT of NOISE AND HEARING

by

Alf Ivarsson and Per Nilsson

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ADVANCES IN MEASUREMENT OF NOISE AND HEARING

by

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University of Lund
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Malmo 1980

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Litos Reprotryck i Malmo AB 1980

To the memory
of
SVEN INGELSTEDT

The present thesis is based on the following papers which will be referred to in the text by Roman numerals:

I. Erlandsson, B., Håkanson, H., Ivarsson, A., Nilsson, P. & Salén, B. Ear-borne sound level dosimeter. *Review of Scientific Instruments 47:1380-1382, 1976*

II. Erlandsson, B., Håkanson, H., Ivarsson, A., Nilsson, P. & Salén, B. Estimation of noise by integration of exposition time exceeding preset sound pressure levels. *Scand Audiol 5:213-218, 1976*

III. Erlandsson, B., Håkanson, H., Ivarsson, A., Nilsson, P. & Salén, B. Calculation of noise dose from time distribution of sound levels. *Acta Otolaryngol (Stockh), Suppl. 366.68-76, 1980*

IV. Erlandsson, B., Håkanson, H., Ivarsson, A., Nilsson, P. & Salén, B. An impulse sound generator and some comparative experiments with different noise dosimeters. *Acta Otolaryngol (Stockh), Suppl 366·77-86, 1980*

V. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P. Comparison between stationary and personal noise dose measuring systems. *Acta Otolaryngol (Stockh), Suppl. 360·105-108, 1979*

VI. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P. Noise dose measurements with stationary and ear-borne microphones. *Acta Otolaryngol (Stockh), Suppl. 366·86-98, 1980*

VII. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P. Comparison of the hearing threshold measured by manual pure-tone and by self-recording (Békésy) audiometry. *Audiology 18 414-429, 1979*

VIII. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P. The accuracy of hearing measurements for detection of hearing impairment caused by noise. *In International symposium on the Protection of Workers against Noise*

International Labour Office, Geneva. (in press)

IX. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P.
The difference in protection efficiency between earplugs and earmuffs.
An investigation performed at a workplace.
Scand Audiol (in press)

X. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P.
The effect of static middle ear pressures on the hearing threshold.
Acta Otolaryngol (Stockh) (in press)

XI. Erlandsson, B., Håkanson, H., Ivarsson, A. & Nilsson, P.
The reliability of Békésy sweep audiometry recording and effects of the earphone position.
Acta Otolaryngol (Stockh) Suppl. 366:99-112, 1980

XII. Erlandsson, B., Håkanson, H., Ivarsson, A., Nilsson, P. & Wersall, J.
Hair cell damage in the guinea pig due to different kinds of noise.
Acta Otolaryngol (Stockh), Suppl 367, 1980

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ADVANCES IN MEASUREMENT OF NOISE AND HEARING

GENERAL INTRODUCTION

The damaging effect of noise on hearing has been known for a long time. Already in 1741, Linneus (von Linné from 1762), professor of practical medicine in Uppsala, Sweden, noted that "Ferrarii, smiths, become hard of

Errata

Acta Otolaryngol, Suppl. 366.

Page 9, line 3. "Martin,1974" *should read* "Martin & Rood, 1974"
Page 18, line 9. *Delete* "(ISO R1999)"
Page 27, line 20. "Lagerhalm" *should read* "Lagerholm"
Page 48, line 10. "Arnolds" *should read* "Arnold"
Page 52, line 10, "Johnson" *should read* "Johnsson"
Page 53, line 12. "Stockwell,1969" *should read* "Stockwell et al,1969"
Page 54, line 3, - " - " " - " -
Page 53, line 24, "(1980)" *should read* "(1980b)"
Page 59, line 5, "Johson & Farin" *should read* "Johnson & Farina"
Page 61, line 34,"Haeman,J.K.,Klain" *should read* "Haseman,J.K.,Klein"
Page 70, line 5, *Delete* "amplitude"

CHAPTER I. EAR-BORNE NOISE DOSE MEASUREMENTS

INTRODUCTION

Damage Risk Criteria (DRC) for regulation of allowable occupational noise exposure have been available for some years and constitute the basis for hearing conservation programs The most widely accepted DRC, the Recommendation R1999 from the International Organization for Standardization (ISO 1971) is based on the equal-energy principle, which postulates that there is a relation between the permanent hearing damage and the total noise energy The equal-energy principle was supported by the studies of Kylin (1960), Burns & Robinson (1970) and subsequent research by Atherley & Martin (1971), Cuberan et al (1971), Martin (1973) and Rice & Martin (1973) suggested the extension of the equal-energy concept to include impulse noise

Industrial noise can be continuously or intermittently distributed and can occur as steady-state, fluctuant or as impulse noise

According to the ISO-document, noise must be measured using an A-weighting filter, constructed to resemble the frequency response of hearing Eighty-five dB(A) is set as the upper limit of daily continuous noise, which is generally accepted as non-injurious Measurement of occupational noise must therefore include both A-weighting and measurement of the total energy during the day Ordinary sound level meters indicating the momentary sound level are sufficient only at continuous steady-state noise When the sound levels vary, noise dosimeters must be used which are capable of continuous registration and integration of the sound energy from which the equivalent sound level, L_{eq}, can be calculated These devices were earlier stationary

One of the first personal pocket-borne dosimeters were described by Lagerholm & Toremalm (1967, 1970) and later on several portable pocket dosimeters have emerged on the market

In some of the major investigations which have contributed to the knowledge of the relationship between occupational noise and hearing damage, the noise measurements were made with the microphone kept close to the worker's ear (Kylin, 1960, Holmgren et al, 1971) Thus, later models of pocket borne noise dosimeters have been devised to permit measurements with the microphone close to the ear It is, however, difficult to measure occupational noise as well as to analyze the importance of the different variables of the measured noise such as frequency, intensity and exposure time, with respect to hearing damage Thus, for example, when using sound level meters, errors in reading

can occur depending on the response time for the meter if the sound contains impulse noise (Wilkerson, 1974) This problem of measuring noise correctly also attends the use of dosimeters (Martin, 1974).

In a series of papers the construction and use of an ear-borne noise dosi- meter is described This new device operates a quite different measuring principle for evaluation of the noise dose, which also is described Finally, are discussed the advantages of measuring noise at the entrance of the ear canal compared to conventional stationary measurements

I. EAR-BORNE SOUND LEVEL DOSIMETER

Commercially available pocket-borne dosimeters record the total energy dose It is not possible to determine whether the noise recorded has been of moderate level and of long duration or of short duration with a high level This paper describes an ear-borne dosimeter that measures the time the noise exceeds preset levels

METHOD AND EXPERIMENTAL DATA

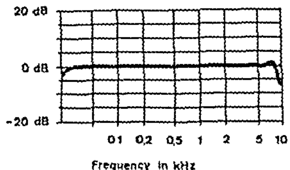
The sound that reaches the ceramic microphone is transformed into an electric signal, which is proportional to the sound pressure The microphone has a flat frequency response to 10 kHz, see fig 1 upper left Close to the microphone is an impedance transformer (FET-amplifier) to transform the high ohmic signal into a signal less sensitive to disturbances

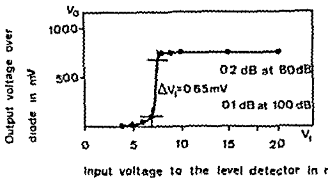
According to international recommendations, the signal is A-filtered With the resistors, R_1 and R_2, the amplification can be adjusted in the subsequent operational amplifier

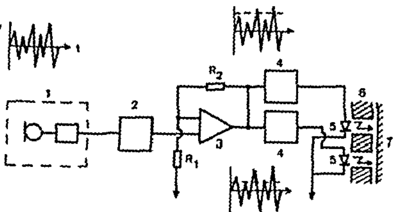
The amplitude of the signal is felt by level detectors When the amplitude exceeds the preset level of the level detector the light emitting diode is switched on and remains on with constant light intensity as long as the amplitude of the signal is high enough When the input signal (V_1) is changed by 0 65 mV, it causes a change in the voltage across the diode (V_0), which is about 80 % of the voltage step, see fig 1 upper right The 0 65 mV shift corresponds to a change in the input sound level of about 0 2 dB at 80 dB SPL and 0 1 dB at 100 dB SPL

The light from the light-emitting diode darkens a spot with a diameter of 2 mm on a photographic film For the total system the response time is determined by the microphone and the weighting network (A-filter) This means that the system can easily handle impulse sound with a duration down to 1 ms Both the diode and the film are enclosed in a cassette The darkness of the spot on the film is a measure of the time the amplitude has exceeded the preset level

The optical density of the exposed spot on the film can be measured by an optical density meter and then transformed to exposure time The latitude of the film corresponds to 3-4 decades of the time In the fig 1 lower right a time range of about 1 to 1 000 s is shown The time range can easily be changed by adjusting the current through the light-emitting diode The time determination within a range of 3 decades can be kept within 5 %







1 Microphone with preamplifier

2 Weighting network

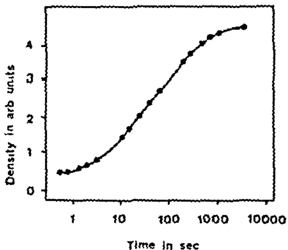
3. Amplifier

4 Level detectors

5 Light emitting diodes

6 Cassette

7 Photographic film



... 1. Block diagram and characteristics of the ear-borne sound level

CONCLUSIONS

The dosimeter can be miniaturized and the complete device will then have the same size as a hearing aid and can be placed in the same way behind the ear with the microphone at the entrance to the ear canal Such a mini-dosimeter includes only two preset level detectors

II. ESTIMATION OF NOISE BY INTEGRATION OF EXPOSITION TIME EXCEEDING PRESET SOUND PRESSURE LEVELS

The Swedish Damage Risk Criterion is based upon the equal-energy principle, which postulates that equal amounts of sound energy have an equally injurious effect and that hearing damage is a function of the total energy that has entered the ear There is empiric support for the validity of this rule for continuous steady-state noise only when the exposure is extended over several years (Botsford, 1970). On the other hand, a lowering of the noise level by 10-23 dB is required when occupational impulse noise is to be taken into account and compared to continuous noise (Passchier-Vermeer, 1971).

One reason for this might be that the energies of occupational impulse noise are located in the frequency range where the damage to the hearing is most prevalent, 3-6 kHz (Bruel, 1976),compared to the continuous noise which has the maximum energies located in a lower frequency region Another reason might be that an impulse sound entering the ear exerts its full power in the inner ear after a response time of 30-100 μs (Bruel, 1976), but the auditory response is detectable at the cortical level after 35 ms (Goldstein & Rodman, 1967) The action of the stapedius muscle is elicited after 15 ms and is maximal after 200 ms This means that the energy of sound impulse reaches the inner ear long before the signal reaches the cerebral cortex and also long before the action of the stapedius muscle is elicited

Another phenomenon which contributes to a subjective false impression is the temporal integration (Pedersen, 1975), which means that sounds of shorter duration than 200 ms are perceived as weaker than the actual sound pressure. This might mean that an impulse with a duration of about 35 μs could be up to 36 dB stronger than the 200 ms long signal and still give the brain the same sense of intensity

Instruments for measuring sound are usually constructed in one of two ways: as peak measuring instruments indicating the maximal sound peak pressure during a measuring period or as RMS (root mean square) instruments indicating the value of a direct current as compared to the alternating sound pressure signal measured To perform this averaging, RC-networks are usually used with different kinds of time constants Most common is the slow circuit with an integration time of 500 ms, which makes it possible to read the meter. However, when this meter-reading is used, information of the peak level is lost Another way of measuring fluctuating or intermittent noise is to measure the noise dose in order to calculate the total equivalent sound level, L_{eq} In this method all details of the sound pressure variations or

peak levels are lost The dilemma is thus that many details of the sound are of interest at the same time but that it is difficult to measure them with the same type of instrument

A new method for doing this is to record the duration (Δt) of the sound pressure wave exceeding preset levels, see fig 2 The sum of all Δt (Σ Δt) is the total time during which a preset sound pressure level has been ex ceeded and from formula $p^2 \times \Sigma \Delta t$ the total noise dose for this level may be calculated When several sound pressure levels are used it is possible to obtain the total equivalent noise level

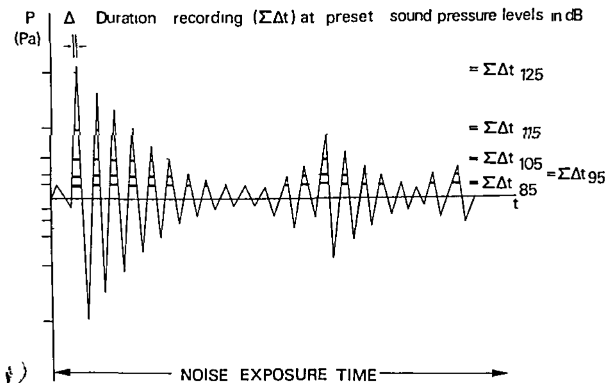


Fig 2 The principle of registration of the exposure time exceeding preset sound pressure levels

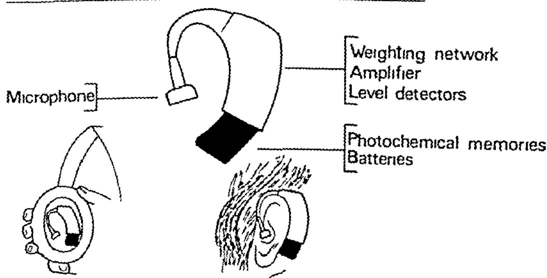
METHODS AND RESULTS

The construction has been described in detail by Erlandsson et al (1976a) and a summary is given in paper I Fig 3 shows the SLD (sound level dosimeter) miniaturized to the size of an ear-borne hearing aid, which makes it possible to use it under most earmuffs The black cassette contains the batteries, the light-emitting diodes and the photo memory The SLD is switched on when the parts are assembled In practice two SLDs, each with two preset sound pressure levels, are used In a first test run the time values were obtained from a worker serving a pneumatic machine The recorded time at the four levels, T_{85}, T_{95}, T_{105} and T_{115}, were tested upon three different assumptions of wave-forms for calculating the L_{eq}-value Table I shows that the differences between the methods of calculation are small If other possible errors are included it should thus be possible to express the L_{eq}-value with an accuracy of ± 2 dB(A)

Table I

Variation of L_{eq} in dB(A) between different waveforms at equal recorded durations

Square waveforms		Triangular waveforms		Sinusoidal waveforms	
$(Pm)^2$	$(P)^2m$	$(Pm)^2$	$(P)^2m$	$(Pm)^2$	$(P)^2m$
96 0	97 0	95 1	95 5	95 3	95 6



F ? Ear-borne sound level dosimeter and its application

In another test SLDs worn by a welder at a shipyard were exposed to the same noise environment. To protect his hearing the worker on the first occasion used earplugs which did not interfere with the two SLDs, on the other occasion the worker used earmuffs which covered also the pair of SLDs The unprotected SLD measurement was L_{eq}= 90.1 dB(A) compared to 88 5 dB(A) by a stationary noise dosimeter B & K 4423 positioned 1.5 m behind the worker The value obtained under the earmuffs was 71.1 dB(A).

CONCLUSIONS

Compared to other sound measuring devices, the SLD enables sounds of different kinds, impulsive as well as continuous, to be recorded for periods during which the pressure peaks exceed preset sound levels The response time is about the same as that of the inner ear, 100 µs. The method thus enables estimation of the distribution of sound energies, peak sound pressures, the total equivalent sound level (L_{eq}) in dB(A) as well as a rough estimate of the non-exposure time below 85 dB(A).

The position of the microphone at the entrance of the ear makes measurement of the actual sound pressure entering the ear canal possible and thus eliminates the disadvantages of other measuring methods concerning the evaluation of baffling effects and distance effects. The device facilitates new possibilities of prospective studies of the individual total daily exposures to all kinds of noise, including impulse noise

III. CALCULATION OF NOISE DOSE FROM TIME DISTRIBUTION OF SOUND LEVELS

The range of sound pressure variations detectable by the human ear is so wide that the amplitudes are most conveniently expressed by a logarithmic scale, the dB scale

The pressure variations of audible sound are so rapid that they must be expressed as mean values to be possible to read When noise is averaged over longer periods the "equivalent sound level", L_{eq}, is a widely used expression in the estimation of noise and for evaluation of the damage risk

This paper compares the usual measurements with an alternative method for calculating and evaluating the sound level

MATERIALS AND METHODS

After recording of the noise, the sound pressure variations are presented in a cumulative curve (fig 4a) showing the total time at each sound pressure level The original paper describes how this curve can be used in two ways for calculating the equivalent sound level

The cumulative curve is then presented in a log-log diagram (fig 4b) and it is shown that the energy content of the sound, which is described by the curve, can be calculated from the time values of each fifth or tenth dB(A) sound pressure level

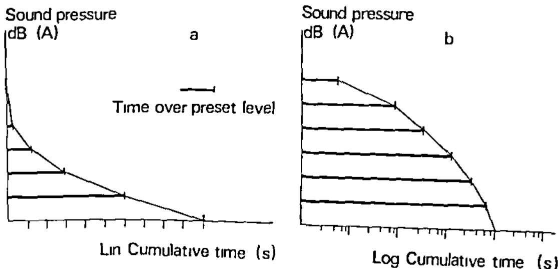


Fig 4 Curves describe the cumulative time at different sound pressure levels
a) Linear distribution of cumulative time
b) Distribution of cumulative time with logarithmic scale

Cumulative sound pressure distributions of the same form as in fig 4b were obtained from 10 measurements of different noise sources with contributions of impulse noise in a machinery factory Different methods for calculating the total sound energy were applied to these curves

RESULTS AND DISCUSSION

Different numbers of sound pressure levels were used in calculating the L_{eq}-value The results are in good agreement with the values obtained by calculations recommended by the International Organization for Standardization. ~~(ISO R1999)~~

CONCLUSION

A method is developed which makes it possible to calculate the usual measures of occupational noise from recordings of the sound duration above certain sound pressure levels The precision of the method enables the use of ear-borne noise dosimeters with only a few preset sound pressure levels

IV. AN IMPULSE SOUND GENERATOR AND SOME COMPARATIVE EXPERIMENTS WITH DIFFERENT NOISE DOSIMETERS

A noise dosimeter should be capable of measuring the equivalent sound level, L_{eq}, for different types of noise, including impulse noise. This report describes an experiment where the L_{eq}-value of impulse noise was measured with three different dosimeters. For this purpose we constructed an impulse sound generator. The results were compared with a L_{eq}-value obtained from an oscilloscope by squaring and integrating the sound pressure over the time.

METHODS

A motor driven hammer was constructed with which it was possible to vary the height of the sound pressure peak, the duration of the pulse, the main frequency content and the repetition rate of the impulse, see fig. 5. The turning rate and the repetition rate of the hammer blows could be varied with aid of a gear box

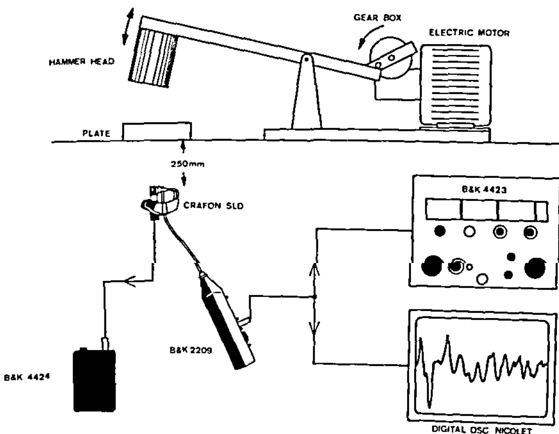


Fig 5 The impulse sound generator and experimental set-up used in evaluating the L_{eq}-response of different noise dosimeters.

The hammer test and the dosimeter experiment were performed in a non-reverberant room The impulse sound pressure was measured with a precision sound level meter, B & K 2209 using an A-filter and equipped with a 1/2 microphone, B & K 4165 The amplified signal was fed to a digital memory oscilloscope, Nicolet 1090 A The vertical axis of the oscilloscope was calibrated in the sound pressure unit, Pascal By squaring and integrating the sound pressure over the time, we obtained a number proportional to the energy content (Pa^2 x s) From this value, L_{eq} can be calculated if we know the repetition rate of the impulse sound generator

The dosimeters employed in this study are shown in fig 5 and are the following Bruel & Kjaer stationary dosimeter, B & K 4423, Bruel & Kjaer pocket-borne dosimeter, B & K 4424, and two ear-borne dosimeters, Crafon SLD, with preset levels of 85, 95, 105 and 115 dB(A) All the fronts of the microphones were placed in the same plane and perpendicularly to the sound direction

R E S U L T S

Reproducibility of the impulse sound generator

It is possible to operate the hammer for more than 72 hours continuously without changes greater than 0 1 dB(A) of the L_{eq}-value At the start and after 50 000 hammer blows there was no difference in the sound pressure pattern during the first 7 ms, where about 85 % of the energy content of the impulse is located

Response of different noise dosimeters

L_{eq}-values, calculated from the oscillogram and values recorded by different noise dosimeters during three test runs with the same repetition rate (25 impacts/s) of the impulse generator, are shown in the table II There was an obvious divergence of the values recorded by the pocket-borne dosimeter, B & K 4424

Table II

Equivalent continuous sound level L_{eq} dB(A)

Test run	1	2	3
Oscillogram	92 4	91 9	90 0
Crafon SLD	92 0	93 0	88 6
B & K 4423	91 3	92 0	90 0
B & K 4424	81 8	82 6	82 2

CONCLUSIONS

An impulse sound generator was constructed. The range of variation of the sound pressure pattern recorded during 10^5 impacts was very narrow. This device was used in evaluating the response of different noise dosimeters. The L_{eq}-value obtained with aid of the new ear-borne dosimeters and that measured with the stationary dosimeter agreed well with the L_{eq}-value calculated from the oscillogram However, the equivalent continuous sound level recorded by the pocket dosimeter was too low when exposed to impulse sound with repetition rates of 1.25 to 2.5 impacts/s. This may be explained by the construction of the dosimeter, which has a time constant of 500 ms and a lower intensity limit of 80 dB(A)

V. COMPARISON BETWEEN STATIONARY AND PERSONAL NOISE DOSE MEASURING SYSTEMS

The usual way to measure occupational noise is with the use of a sound level meter Problems in the measurement of fluctuating or impulse noise can be solved by the use of dosimeters The disadvantage of stationary measurements, when personal noise dose is to be estimated (Martin, 1970, Brammer & Piercy, 1977) can be overcome with personal noise dosimeters, which may, however, have problems to register impulse noise properly (Svensson, 1978)

The aim of this study was to explain why large differences in noise doses can occur in the recordings made in apparently the same noisy situation

MATERIALS AND METHODS

Three different types of sound level dosimeters were used (1) Bruel & Kjaer (B & K) 4423 stationary noise dose meter, (2) B & K 4424 pocket-size personal noise dosemeter, (3) Crafon SLD ear-borne dosimeters

Three series of measurements were performed The first series was a stationary test comparing a) five Crafon SLD with preset levels differing 5 dB and covering the measuring range from 85 to 130 dB(A), b) two standard Crafon SLD with the usual preset levels of 85, 95, 105 and 115 dB(A), c) B & K 4423 and d) B & K 4424 In the second series the noise exposure of 10 subjects was measured on 5 occasions with two Crafon SLD, one on each ear, and a pocket-borne B & K 4424 Discrepancies between the measurements of 4424 and Crafon SLD in two welders working with carbon arc gouging initiated a detailed investigation, which was rformed in the third series When performing carbon arc gouging a worker equipped with a standard set of two SLD, one on each ear Two pocket dosimeters B & K 4424, one with the microphone mounted on the helmet, were also used, besides which the signal from a microphone held 30-80 cm from the ear at shoulder level was fed to the stationary B & K 4424 dosimeter

RESULTS AND DISCUSSION

In the first series there was a good agreement between all the stationary test measurements, indicating that both the Crafon SLD and B & K 4424 have a comparable accuracy The difference between the L_{eq}-values calculated from Crafon SLD in 5 dB steps and the standard Crafon SLD in 10 dB steps did not exceed 0 7 dB in any of 5 test runs with stationary doses around 90 dB This

supports the assumption by Erlandsson et al (1976b) that four preset levels are enough for calculating the L_{eq} in this noise environment In the second series, the results of 50 comparative measurements demonstrated that for most work processes the Crafon SLD gives an L_{eq}-value about 2 dB higher than the value measured at the site of the breast pocket This may be due to the repetition rate of the impact sounds (see paper IV) The discrepancies during carbon arc gouging were analysed in the third series and the results are given in table III and fig 6

Table III

L_{eq}-values in dB(A) obtained at carbon arc gouging

Dosimeter	Crafon SLD	B & K 4424	B & K 4424	B & K 4423
Microphone position	Ear canal and entrance	Helmet	Breast pocket	30-80 cm from the ear
L_{eq}-value	107 8	113 4	117 5	111 0

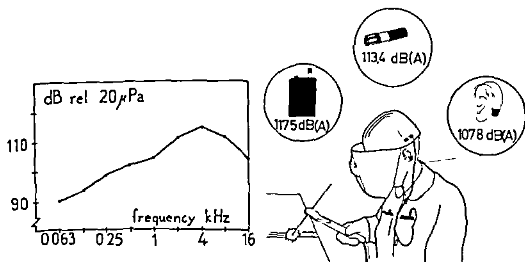


Fig 6 Carbon arc gouging To the left frequency analysis of noise is given and to the right the L_{eq}-values measured with noise dosimeters at different microphone positions

The difference between the values of the breast-pocket microphone and the helmet microphone of the two B & K 4424 might be explained by the difference in distance from the sound source The same explanation seems to be possible for the difference between the value of the helmet microphone and the stationary dosimeter The Crafon SLD showed considerably lower values Frequency analysis of the noise from carbon arc gouging revealed that most of the energy was centered around 4 kHz (1-16 kHz) The difference between the measurements by Crafon SLD and the other dosimeters could be explained by the large proportion of high frequency components of the sound, to which small changes in the position of the microphone may cause great changes in the dose value In this case the difference was caused by the welder's visor

C O N C L U S I O N S

Comparative dose measurements showed the practical usefulness of different kinds of noise dosimeters Discrepancies between different noise dosimeters may occur, and such a situation was closely analysed From the analysis the conclusion can be drawn that in investigations of the correlation between personal noise dose measurements and hearing impairment the dose should be measured with the microphone at the entrance to the ear canal

VI. NOISE DOSE MEASUREMENTS WITH STATIONARY AND EAR-BOPNE MICROPHONES

In order to estimate the hazards of occupational noise-induced hearing loss, the noise has for many years been measured mainly with stationary equipment In recent years personal noise dose measurement has been more frequently used This has focused interest on the problem of different microphone positions in the two types of measurement

The aim of this study was to compare the results obtained with a stationary microphone and a mobile, ear-borne microphone Simultaneous recordings were made for different occupations and working positions in order to find out whether systematic differences could be found

EQUIPMENT AND METHOD

Two identical, parallel channels were used, one for the stationary and one for the mobile registration The microphone of the mobile unit was placed near the entrance of the ear canal and the signals from both microphones were fed to a two-track tape recorder All measurements were performed in the same workshop and 10 workers of different occupations and working positions served as test subjects The frequency spectrum analysis was performed in the laboratory with an octave filter in conjunction with a stationary dosimeter and with a Fast Fourier Transform (FFT) analyzer

RESULTS AND DISCUSSION

The equivalent noise doses, obtained from the stationary recordings, agreed with earlier stationary measurements (Oxelbeck & Elmegren, 1973) The noise doses measured close to the ear were also consistent with earlier measurements obtained with an ear-borne sound level dosimeter, Crafon SLD, (Erlandsson et al, 1976a,b, 1980a) The discrepancies between noise dose measurements from the stationary and ear-borne microphones were large and the discrepancies increased in almost all recordings with the frequency, which is in agreement with the findings of other authors (Muldoon, 1973, Kuhn & Burnett, 1977, Olsen & Carhart, 1975)

Both the octave anlysis and FFT-analysis revealed a relatively low energy content at lower frequencies This explains why A-weighting (the filter function for evaluation of occupational noise, recommended by International Organization for Standardization ISO R1999) reduces the total noise dose by

only about 1-2 dB.

The most important finding, however, was that no systematic or recurrent differences could be found between the doses measured close to the ear and those measured with stationary equipment for persons with the same occupation or the corresponding mobility or positions with the same reverberant field

CONCLUSIONS

There is a considerable difference between the noise doses from stationary and ear-borne microphone positions but these differences vary with occupation, mobility of the test subject and reverberant field in the working position in such a way that it does not seem possible to find any correction factors to enable comparison of occupational noise recorded with stationary and ear-borne microphones. This means that in hearing conservation programs the noise must be measured at the entrance to the ear canal.

*)

GENERAL DISCUSSION

Occupational hearing loss is caused by a complex interaction of all the variables of noise, i e frequency, intensity and duration An assessment of the exposure to occupational noise for hearing conservation purposes has been presented by the International Organization for Standardization in their recommendation ISO R1999 In this document some fundamental assumptions have been made

1 The sound level should be determined at the position occupied by listener's ear (preferably with the listener absent)
2 Impulse noise is to be regarded only as such when occurring as series of noise bursts

We discussed and tried to elucidate these assumptions Several investigations bearing on this subject (Burns & Robinson, 1970, Guberan et al, 1971, Passchier-Vermeer, 1971, Martin, 1973, Rice & Martin, 1973, Bruel, 1976) prompted us to study the problems of noise measurements and their relation to occupational hearing loss

The desire expressed in the international recommendation, followed up in the Swedish recommendation SEN 590111, to measure noise at the same position at the ear in workers with high mobility seems to have contributed to the development of personal noise dosimeters (Lagerhölm & Torenalm, 1967, 1970) These pocket borne dosimeters have achieved a wide use in recent years (Kihlman et al, 1973, Christiansen et al, 1975, Nilsson et al, 1976) Problems concerning the accuracy of measurements and especially the interpretation of impulse noise are, however, frequent (Martin & Pood, 1974, Mellot, 1978, Svensson, 1978)

The growing need for personal noise dose measurements and also the need to measure the sound at the entrance to the ear initiated our work with the development of the ear-borne sound level dosimeter The construction of the dosimeter is described in paper I and its application in paper II

A cumulative analysis of the sound pressure distribution, i e a diagram giving the total time certain preset sound levels have been exceeded at each appropriate level,forms a very convenient basis for further calculation of the energy mean level, the L_{eq}-value as well as the average sound pressure level These variables are determined by simple calculations using areas found in the diagram (papers II and III) This is a very accurate method for calculating the noise dose when compared to the more traditional method

of electronical squaring and averaging. The accuracy is, however, a function of the number of sound pressure levels involved in the calculations. But investigations of sound pressure level distributions obtained in a machine factory show that also a limited number of levels give a satisfactory value of the equivalent sound level. The accuracy depends mainly on the frequency response of the microphone and only to some extent on the subsequent electronics, which contrasts with traditional noise dosimeters where electronic treatment of the microphone signal is important in the estimation of the over-all accuracy of the noise dose.

This calculation method was utilized in the small ear-borne noise dosimeter described above, which uses only four levels and gives a fairly good value of the equivalent sound level.

The assumption that the noise should be measured at the position of the listener's ear has certain implications on the relation between stationary and mobile (near the entrance to the ear canal) noise dose measurements and was investigated in paper VI. With the use of precision instrumentation it was shown that there is no simple relation between a stationary and a mobile noise dose measurement. Consequently there is no factor which translates the noise level of a stationary measurement into a reliable value for the actual sound level at the entrance to the worker's ear in the actual workshop. The same conclusion is also drawn in paper V. This may be compared to some earlier works by Kylin (1960), Burns & Robinson (1970) and Holmgren et al (1971) It is evident that also these authors have been aware of the measuring problem and therefore made the noise measurements with a microphone close to the worker's ear. Brammer & Piercy (1977), who used a miniature microphone in the cavum of the concha were able to transform the measured sound pressure levels from the cavum of the concha to those at a center-head position in order to relate the achieved values to present recommendation for damage risk evaluation.

IN CONCLUSION, it appears that in the estimation of the risk of hearing impairment by occupational noise, the noise should preferably be measured at the entrance to the ear canal We therefore constructed a noise dosimeter filling this requirement

The dosimeter

- has an accuracy better than $\pm$ 2 dB,
- does not weigh more than an ear-borne hearing aid,
- does not disturb the wearer,
- gives values from which the L_{eq} and the mean sound pressure level can be calculated, and
- can be worn under earmuffs

CHAPTER II. HEARING MEASUREMENTS FOR DETECTION OF HEARING IMPAIRMENT CAUSED BY NOISE AND FOR ASSESSING EFFICIENCY OF HEARING PROTECTORS

INTRODUCTION

Next to presbyacusis, noise is the most common cause of hearing impairment (Surján et al, 1973) People working in noisy environments should therefore have their hearing regularly checked (OSHA, 1974, Austr. Stand. Code Practice, 1976, OECD, 1978) The hearing threshold can be measured if the test subject indicates the lowest sound intensity he hears from tones of different frequencies. The results of such an examination can then be given in a chart, an audiogram, as hearing threshold levels or hearing loss for pure tones at specified frequencies. The appearance of the audiogram gives the examiner an impression of the character and site of the hearing loss. Regular exposure to industrial noise may cause damage to the hair cells of the inner ear, which results in a permanent hearing loss most often in the frequency range of 2 to 8 kHz (fig 7). Hearing aids can only partially compensate for such a loss. Noise is also known to cause a temporary hearing loss as well as ringing in the ear, i.e. tinnitus

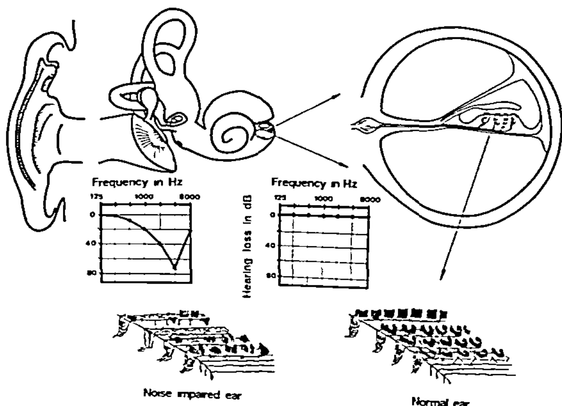


Fig 7 Schematic figure illustrating hearing loss and hair cell damage for a noise impaired ear and a normal ear.

The progress of hearing loss due to industrial noise is slow and will not offer any practical problem before some 20 years of such exposure (e.g Gallo & Glorig, 1964; Taylor et al, 1965). The first sign of such impairment is poor discrimination of speech in more or less crowded environments, i e in the presence of background noise. With increasing hearing impairment discrimination will be difficult also in a quiet environment. Thus, occupational impairment of hearing is a major problem and preventive measures are urgent

It has hitherto been the rule to use repeated manual pure tone audiometry in anti-noise projects all over the world. In pure tone audiometry an operator manually tries to ascertain hearing thresholds according to a statistical method. However, the accuracy of the hearing thresholds found by this method is limited by low precision of the measurements. Thus, the uncertainty in determining the hearing thresholds prevents early detection of hearing impairment due to noise.

The main purpose of this part of our study was to find out whether it is possible to verify hearing impairment caused by noise earlier by means of repeated Bêkesy sweep audiometry. Early detection of such a hearing impairment would make it possible to prevent progress of the impairment by transferring the workers to less noisy working places. Finally, a more precise determination of the hearing threshold would also make it possible to check and compare the efficiency of different kinds of hearing protectors.

VII. COMPARISON OF THE HEARING THRESHOLD MEASURED BY MANUAL PURE-TONE AND BY SELF-RECORDING (BÉKÉSY) AUDIOMETRY

In our studies of occupational hearing loss at Kockums Shipyard, Malmo, Sweden one purpose was to find out if it is possible to confirm early changes in hearing thresholds due to noise exposure. For this it was necessary to require knowledge of the precision of the methods used for determining the hearing thresholds.

The aim of the present study was to evaluate the reliability of, and the relation between, hearing thresholds found by manual pure tone and self-recording Békésy sweep audiometry in workers with a varying degree of hearing impairment.

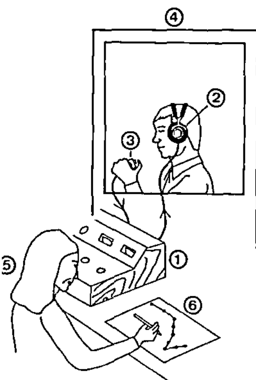
MATERIAL AND METHODS

The hearing thresholds of 115 male workers, aged 25 to 63 years, at Kockums Shipyard were determined both by conventional manual pure tone audiometry and self-recording Békésy sweep audiometry. All determinations were made at least 16 hours after the persons had been exposed to occupational noise. The pure tone audiometry was performed by the same audiometrician, see fig. 8. By increasing the tone intensity in 5 dB steps the lowest level of the tone, at which the test subject could hear two out of three tone bursts, was determined. This test was done three times and the lowest (better) tone level was taken by the audiometrician as the level of the subject's hearing threshold at each test frequency When the hearing threshold was tested with the Békésy sweep audiometer, the subject himself recorded his hearing threshold during a frequency sweep from 250 to 10 000 Hz and back to 250 Hz, see fig. 9 . The attenuation rate was 2 5 dB/s with pulsed tone-presentation and the duration of the sweep from 250 to 10 000 Hz was 400 s. The hearing threshold was calculated as the mean value between the upper peaks of the tracings representing the moments where the subject could no longer hear the tone, and the lower peaks, where the subject could just hear the tone.

RESULTS

Comparison between hearing thresholds determined with pure tone and Békésy sweep audiometry

The hearing threshold was determined for 230 ears at each test frequency. The Bekesy method gave the lowest values. The regression equation for Békésy threshold (HL_B) on pure tone threshold (HL_T) for the test frequencies 1-6 kHz was: $HL_B = -3.4 + 0.93 \times HL_T$ and for pure tone on Békésy $HL_T = 8.0 + 0.96 \times HL_B$.



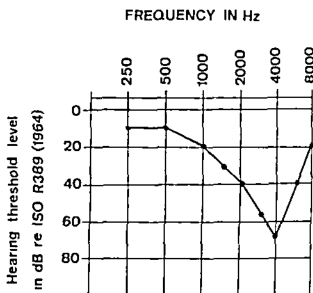
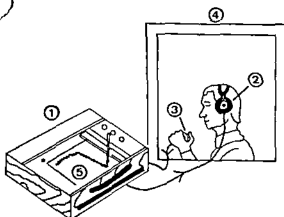


Fig 8. Manual pure tone audiometry. 1=Audiometer, 2=Earphone, 3=Response switch, 4=Sound-proofed booth, 5=Audiometrician and 6=Audiogram chart showing hearing threshold levels at the frequencies 0 25 to 8 kHz tested in 5 dB-steps



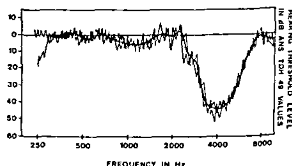


Fig 9. Self-recording Bekesy sweep audiometry 1=Békésy sweep audiometer, 2=Earphone, 3=Response switch, 4=Sound proofed booth and 5=Graphic frequency sweep recording from 0 25 to 10 kHz and back to 0 25 kHz and calculated mean hearing threshold level within the frequency range.

Reliability of [illegible] audiometry

Ten subjects were studied five times at intervals of at least 24 hours. The pooled estimate of the variance of the hearing thresholds was computed by calculating first the variance for each ear and then the mean value of the variance for all 20 ears. The standard deviations have their lowest values for 1 kHz, increasing slowly towards lower and higher frequencies, see table IV.

Reliability of pure tone audiometry

With the aid of a formula by Madansky (1959), it is possible to calculate the standard deviation of the pure tone threshold values provided the standard deviation of the Békesy threshold values and the regression equation for a pure tone on Békésy threshold at the different audiometric frequencies are known. The standard deviations of the hearing thresholds in pure tone audiometry are about twice as large as those obtained in a Békésy recording, see table IV.

Table IV
Standard deviation of hearing threshold in dB at different frequencies in kHz

Frequency	0 5	1	1 5	2	3	4	6	8
Bekésy	3.2	2 4	2 8	3.5	2.8	3.3	3 4	4.2
Pure tone	6 3	6.2	6 5	4 7	6.6	5.3	7.3	10.4

C O N C L U S I O N S

The comparison between the two types of audiometry shows that Békésy sweep audiometry gives a lower (better) and more reliable hearing threshold. It is possible to establish a linear relation between pure tone on Bêkesy hearing threshold and vice versa at the different audiometric frequencies. As the precision of self-recording Békesy sweep audiometry has shown to be higher than that of manual pure tone audiometry, this method will make it possible to detect and confirm a progress of hearing impairment caused by exposure to noise earlier

VIII. THE ACCURACY OF HEARING MEASUREMENT FOR DETECTION OF HEARING IMPAIRMENT CAUSED BY NOISE

Most Damage Risk Criteria relating noise levels with the degree and rate of the hearing loss are based on results obtained by pure tone audiometry. The accuracy of these methods is limited for several reasons and in paper VII we showed, that the standard deviation of hearing thresholds determined by a conventional pure tone audiometry was about twice as large as that obtained by a Bēkēsy sweep audiometry.

In Hearing Conservation Programs, where the interest is focused upon the progress of the hearing loss, the precision of the method measuring the hearing is important and for this purpose Bekēsy sweep audiometry seems to offer an advantage.

The aim of this study was to find out whether an even higher reliability of hearing threshold measurement in subjects with noise-induced hearing loss can be achieved from the summed hearing thresholds between 2 and 8 kHz, HT_{2-8}, in a Bēkēsy sweep recording The method was applied in a 2-year follow-up study of workers at Kockums Shipyard.

MATERIALS AND METHODS

Bekēsy sweep audiometry with pulsed tone presentation and an attenuation rate of 2 5 dB/s sweeping from 0 25 to 10 kHz and back to 0.25 kHz was used. The hearing threshold was determined as the mean value between the upper and lower peaks of the Bekesy sweep recordings. This test was made after avoidance of occupational noise for 16 hours in shipyard-workers aged 25-63 years. The growth of the hearing loss as a function of age and duration of noise exposure was greatest in the range of 2-8 kHz, which is in accordance with other investigations

RESULTS

Reliability of summed hearing threshold between 2 and 8 kHz

A Bēkēsy sweep audiogram of 10 subjects with different noise-induced hearing impairment was performed five times at 1-day intervals From 9 subjects (18 ears) the summed hearing threshold value for each integer frequency from 2 to 8 kHz, HT_{2-8}, could be calculated, and the standard deviation was found to be 17.1 dB.kHz This gives an average standard deviation for each integer kHz between 2 and 8, which is smaller than that, which is found at the respective frequencies, see table V

Table \

Standard deviation of hearing threshold in dB at different frequencies in kHz

Frequency	2	3	4	5	6	7	8	HT_{2-8}	Average for integer kHz (2-8)
SD	3.5	2.8	3.3	3.4	3.4	3.8	4.2	17.1	2.5

The summed hearing threshold [illegible]es, HT_{2-8}, of shipyard-workers within two years

Békésy sweep audiograms were obtained of 156 ears in 80 shipyard-workers from two workshops in 1977 and 1979. The stationary noise doses in the boiler shop varied between 89 and 90 dB (A) and in the assembly shop between 84 and 89 dB (A). The individual noise doses were higher when measured at the ears of the workers compared to the stationary noise doses. Almost all of the workers tested had used some type of hearing protectors.

In order to estimate the significance of the hearing loss caused by exposure to noise, the difference in the calculated HT_{2-8} for each ear was corrected for presbyacusis according to the age-correction coefficients of Burns & Robinson (1970) for frequencies up to 6 kHz and of Hinchcliffe (1959) for higher frequencies In 36 % of the ears the increase of the HT_{2-8}-value between 1977 and 1979 exceeded the value of the standard error of the difference between two measurements, 24 dB.kHz, accepted as border value in the HT_{2-8}-measurements (table VI). Eight out of ten unprotected ears showed a significant increase of HT_{2-8}

Table VI

Workshops	Ears	Workers
Boiler shop	35 % (28/80)	52 % (22/42)
Assembly shop	37 % (28/76)	53 % (20/38)
Total	36 % (56/156)	52.5 % (42/80)

C O N C L U S I O N S

The results of this study showed that the reliability could be improved by adding the hearing thresholds for each integer frequency from 2 to 8 kHz in the Bekésy sweep recordings We found that about 50 % of the shipyard-workers studied seemed to have developed significant hearing impairment of at least one of the ears within two years, though most of them had used some type of hearing protector The Bekésy sweep audiometry with calculation of HT_{2-8} seems to improve the possibility to detect progress of hearing loss and also to check the efficiency of hearing protectors.

IX. *THE DIFFERENCE IN PROTECTION EFFICIENCY BETWEEN EARPLUGS AND* EARMUFFS, AN INVESTIGATION PERFORMED AT A WORKPLACE

Analyses of Békésy sweep audiometry showed that within a period of two years about 50 % of the workers studied at Kockums Shipyard had a significant noise-induced hearing loss in at least one of the ears in the frequency range of 2 to 8 kHz despite the use of hearing protectors. Most of the workers had used either earplugs or earmuffs for 5 to 10 years and had also been working all the time in the same noisy environment. It was therefore possible to group the workers as 'plug-men" and "muff-men".

The aim of this study was to compare the hearing thresholds of the two groups in order to assess the difference, if any, between the efficiency of earmuffs and earplugs. Three studies using Békésy sweep audiometry were performed: two retrospective and one prospective concerning threshold changes within an interval of two years.

MATERIAL AND METHODS

The material was selected from two workshops at the shipyard, viz the assembly shop (67 male workers), where segments of a ship are put together, and the boiler shop (55 male workers), where smaller parts of the machinery of a ship are manufactured Most of the workers in the two workshops are platers and welders The equivalent noise doses for stationary measurements vary between 84 and 89 dB (A) in the assembly shop and between 89 and 90 dB (A) in the boiler shop

The ears were otologically examined in connection with the hearing measurements in 1977, 1978 and 1979, and detailed notes were made of the use of hearing protectors The criteria of Burns & Robinson (1970) were used for classifying an ear as normal After avoidance of occupational noise for 16 hours the hearing thresholds were determined with Békésy sweep audiometry. An attenuation rate of 2 5 dB/s with pulsed tone presentation from 0.25 to 10 kHz and back to 0 25 kHz was used The hearing threshold was determined as the mean value between the upper and lower peaks of the Bekésy sweep recordings. The hearing thresholds for each ear were corrected for presbyacusis according to the age-correction coefficients of Burns & Robinson (1970) for frequencies up to 6 kHz, and of Hinchcliffe (1959) for higher frequencies.

Different types of hearing protectors have been used for many years at Kockums Shipyard. In 1948 the first earplugs (glass down) were introduced and earmuffs in 1961. In the table VII the distribution of the various hearing protectors

among the workers in 1977 is given

Table VII

Workshops	Plugs	Muffs	Muffs + Plugs	Unprotected
Assembly shop	38(57 %)	23(35 %)	2(3 %)	4(5 %)
Boiler shop	20(42 %)	19(40 %)	4(8 %)	5(10 %)

RESULTS

Measuring of individual noise dose

Twenty workers in the assembly shop and 20 in the boiler shop were fitted with ear-borne dosimeters, Crafon SLD. The range of the equivalent noise doses ($L_{eq(8h)}$) for welders and platers is given in table VIII. The individual noise dose for welders and platers was higher, when it was measured at the entrance of the ear canal than measured by ordinary stationary instruments.

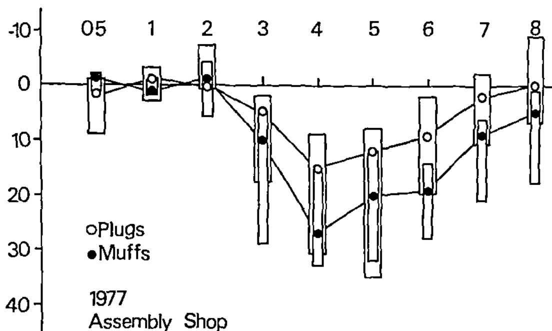
Table VIII

Workshops	Welders	Platers
Assembly shop	91 - 95 dB (A)	91 - 96 dB (A)
Boiler shop	94 - 96 dB (A)	93 - 96 dB (A)

Difference in hearing threshold between "plug-men" and "muff-men"

The hearing thresholds of workers in 1977 and 1978. Eight platers or welders (16 ears), aged 31 to 40 years, wearing either plugs or muffs, were selected from each workshop The duration of employment at the shipyard, years in the same workshop and years during which hearing protectors had been used, were almost the same for "plug men" and "muff-men". The median values for the hearing thresholds corrected for presbyacusis for assembly shop workers and boiler shop workers are shown in fig 10 . The median hearing thresholds were higher for "muff-men" compared to "plug-men" for frequencies above 2 kHz in the assembly shop This difference was not observed in the boiler shop Almost the same pattern was obtained in the hearing test in 1978, which indicated a better protection for "plug-men"

The summed hearing thresholds, HT_{2-8}, of workers in 1977 and 1978. From each workshop were selected matched pairs of "plug-men" and "muff-men", aged 25 to 63 years, of nearly the same age, with nearly the same time of employment and years during which either plugs or muffs had been used as well as years in the



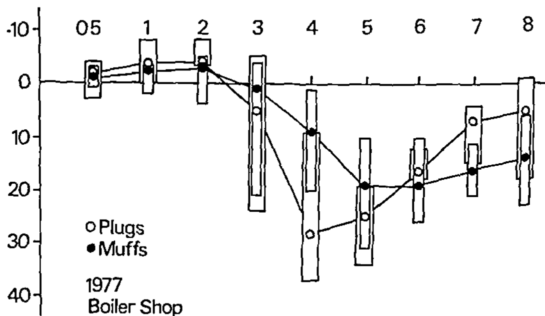


Fig. 10. Median hearing threshold of "plug-men" and "muff-men", aged 31 to 40 years, from two working places at Kockums Shipyard. The diagrams also show the 25 to 75 % ranges which more or less overlap for "plug-men" and "muff-men".

same workshop and occupation. Twelve matched pairs of workers were obtained from the assembly shop and 10 from the boiler shop. The sum of the hearing thresholds corrected for presbyacusis for each integer frequency from 2 to 8 kHz, HT_{2-8}, of each ear was compared within the pair. Most pairs of the assembly shop "muff-men" had greater hearing impairment than the "plug-men". In the assembly shop, there was a significant difference of HT_{2-8} (< 5 %) between workers wearing muffs and workers wearing plugs at the hearing tests in 1977 as well as in 1978 Such a significant difference was not observed in the boiler shop

The shift of the summed hearing threshold (HT_{2-8}) between two years, 1977 and 1979. The calculated difference in HT_{2-8} for each ear was corrected for presbyacusis. The mean difference of HT_{2-8} for workers in each workshop, aged 25 to 63 years, who had used either muffs or plugs for the last 3 years, was compared and the results are shown in table IX . In the assembly shop the difference of HT_{2-8} between "muff-men" and 'plug-men" was significant on the 7 % level. In the boiler shop there was no significant difference between the two categories.

Table IX

Mean and standard deviation of HT_{2-8}-shift between 1979 and 1977 in dB.kHz

Workshops	"Muff-men"	"Plug-men"
Assembly shop	8 38 $\pm$ 5.25 (21 ears)	-1.52 $\pm$ 4.42 (30 ears)
Boiler shop	12.82 $\pm$ 5 87 (17 ears)	15.89 $\pm$ 4.57 (28 ears)

CONCLUSIONS

The hearing thresholds of the shipyard-workers using either earplugs or earmuffs were compared retrospectively in 1977 and 1978 and prospectively from 1977 to 1979 The Békesy threshold measurements showed greater hearing impairment and hearing threshold shifts for "muff-men' than for plug-men" in the assembly shop The individual noise doses at the entrance of the ear canal were almost the same for the workers in the two working places, but the background noise, measured with stationary instruments was found more intermittent in the assembly shop than in the boiler shop. The difference in hearing impairment between the two groups might be explained by the possibility that assembly shop workers remove the muffs more frequently than the plugs in their intermittent noise environment, since it is much easier to remove muffs than plugs. The reduction of the protection efficiency of

earmuffs in relation to the time they were used in the same noise environment is given in fig. 11 This is in accordance with the observation that about 50 % of the workers studied at Kockums Shipyard had a significant hearing loss in the frequency range of 2 to 8 kHz within a period of 2 years despite the use of hearing protectors, see paper VIII

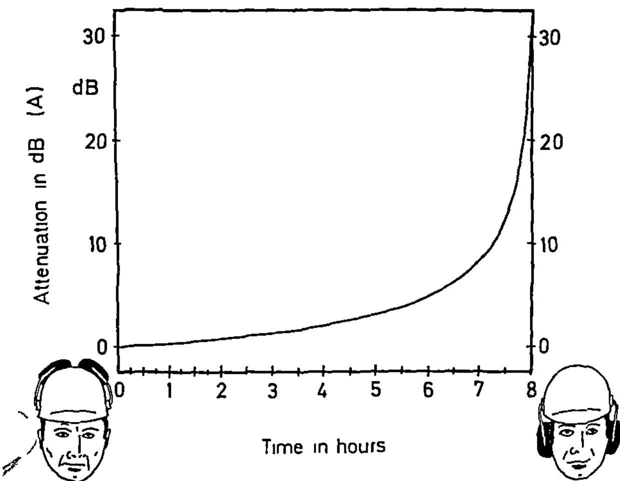


Fig 11 Equivalent protector attenuation in dB(A) during eight hours in the same noise environment in relation to the time the earmuffs covered the outer ears

X. THE EFFECT OF STATIC MIDDLE EAR PRESSURES ON THE HEARING THRESHOLD

The gas enclosed in the middle ear cavity is continuously absorbed, which induces a relative underpressure if the Eustachian tube is kept closed. In ears with a perfect equilibration capacity of the tube, the middle ear pressure will be equal to the ambient pressure, while in ears with less good equilibration capacity of the tube, different pressure gradients in relation to ambient pressure are found (Ingelstedt & Ivarsson, 1978)

The aim of the present investigation was to assess the effect on the hearing thresholds of different middle ear pressure levels and to find out whether the hearing thresholds between 2 and 8 kHz can be measured more precisely by means of Békésy sweep audiometry, when the middle ear pressure is checked.

MATERIAL AND METHODS

Six ears of four selected subjects could completely equilibrate the middle ear pressure with defined levels of relative underpressure in the rhinopharynx, produced by a pressure generator The pressure equilibration was checked by tympanometry. The hearing threshold was recorded by Békésy audiometry with attenuation rate of 2.5 dB/s and pulsed tone presentation at fixed frequencies. The hearing threshold before and after pressure equilibration was determined as the mean value between the upper and lower peaks of the Békésy recording. We used two linear regressions for calculating the shift of the hearing threshold caused by the different levels of middle ear pressure, see fig.12.

Another investigation in 95 shipyard-workers (187 ears) was performed, where the middle ear pressure was calculated from a tympanogram immediately before the hearing test

RESULTS

Effect of variation of middle ear pressure on hearing thresholds

The threshold shifts in dB produced by the relative underpressure in the middle ear of 5, 10 and 15 cm H_2O were recorded for 6 ears at frequencies of 0.5, 1, 2, 4 and 6 kHz The data are presented in fig 13 , where positive values denote threshold losses (poorer thresholds), and negative values, threshold gains (better thresholds) Threshold losses were recorded at 0.5, 1 and 4 kHz and threshold gains at 2 and 6 kHz. At all test frequencies the threshold losses and gains were increased with higher relative underpressure in the middle ear cavity

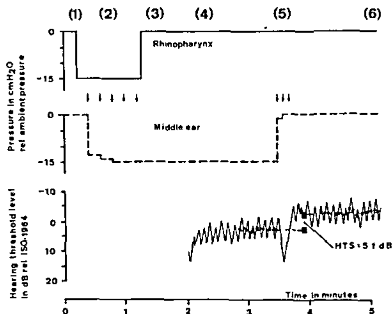


Fig. 12. Recording example (1-6) for measuring a hearing threshold shift (HTS) at a relative underpressure equilibration in the middle ear of 15 cm H_2O at a test frequency of 0.5 kHz. The interrupted curve is an earlier recording of the middle ear pressure by tympanometry during equilibration of rhinopharynx pressure. Arrows = Deglutitions. 1=Pressure change in the rhinopharynx, 2=five deglutitions, 3=subject is placed inside the sound-proof booth, 4=Békésy test is started, 5=three deglutitions for equilibration of the middle ear pressure and 6=Békésy test is stopped.

Fig. 13. Hearing threshold shifts in dB at relative underpressures in the middle ear recorded in six normal ears. The bold line denotes the mean hearing threshold shifts.

Middle ear pressure in shipyard-workers

In 171 ears (91 %) the measured middle ear pressure was close to the ambient pressure In 16 ears (9 %) the relative underpressure in the middle ear was $\geq$ 5 cm H_2O. Most of these ears with $\geq$ 5 cm H_2O were retested after about one month and then the middle ear pressure was different in such a way that, according to the previous experiment, a change in hearing threshold could be expected. The variation in the middle ear pressure was not so pronounced in workers, who had an initial underpressure < 5 cm H_2O relative to the ambient pressure

C O N C L U S I O N S

The results of this study show that a relative underpressure in the middle ear cavity affects the hearing threshold with both hearing loss and hearing gain. The threshold loss was most prominent for 0 5 and 1 kHz and less for 4 kHz. A threshold gain was shown for 2 and 6 kHz At all test frequencies the threshold shifts were increased with higher relative underpressure in the middle ear In order to evaluate the hearing impairment caused by noise, it is thus important to check the middle ear pressure before hearing is tested. A small change in the middle ear pressure (< 5 cm H_2O) relative to the ambient pressure can be ignored by using the summed hearing thresholds between 2 and 8 kHz

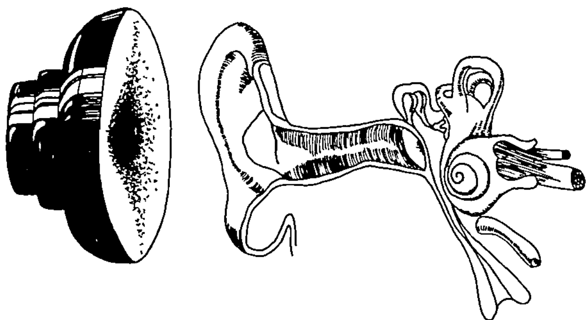


Fig. 14. TDH49P earphone with MX-41/AR cushion for application to outer ear.

Fig. 15. TDH49P earphone with ear speculum inserted in external ear canal.

GENERAL DISCUSSION

A campaign against noise-induced hearing loss includes not only efforts to detect significant changes in hearing threshold as soon as possible, but also to prevent the progress of such a loss. This requires high reliability of the hearing tests used for determining hearing threshold shifts The Swedish National Board of Occupational Safety and Health (1976) recommend repeated hearing tests by manual pure tone audiometry within 6-month to 3-year intervals. This technique is also most often used in other countries. The investigations presented in this thesis have shown that standard deviations of hearing threshold measurements in subjects with different degrees of hearing impairment, determined by a conventional pure tone audiometry and by the same operator, are about twice as large as those obtained by Békésy sweep audiometry. The standard deviations of the pure tone thresholds obtained in our investigation are in good agreement with the results obtained by Brown (1948), but higher than those obtained by Kylin (1960). The improved reliability of hearing threshold measurements by Békésy sweep audiometry was about the same as that which Rudmose (1963) and Delany (1970) found by Békésy audiometry at fixed frequencies Burns & Hinchcliffe (1957), Harris (1964), Delany et al (1966) and Knight (1966) have also shown that Békésy audiometry gives lower (better) hearing thresholds than those obtained by pure tone audiometry. Our investigation gave similar results and showed that a linear relation can be obtained between pure tone and Békésy hearing threshold measurements in persons with different degrees of hearing impairment. Thus, the hearing threshold values obtained by pure tone audiometry can be converted to values obtained by Békésy audiometry and vice versa in the assessment of noise-induced hearing loss

During increasing hearing loss with advancing age and duration of exposure to noise, the growth of the hearing loss is greatest in the range of 2 to 8 kHz. We found that the summed hearing thresholds between 2 and 8 kHz, HT_{2-8}, gave a more reliable expression for the hearing loss shifts. The average standard deviation for integer frequencies in kHz from 2 to 8 kHz is smaller than the standard deviation for each frequency. Similar results have also been obtained by Burns & Robinson (1970) using non-exposed subjects. In our investigation the reliability of HT_{2-8} showed no correlation with the degree of hearing impairment and was not referred to the subject, but to the individual ear.

Burns & Hinchcliffe (1957) and Robinson & Whittle (1973), who used repeated Békésy audiometry, have shown a hearing threshold improvement of 1 to 2 dB at most frequencies within one week and within 15 months, respectively. This

improvement has been ascribed to a learning effect. However, our results showed no trend towards an improvement of the summed hearing thresholds between 2 and 8 kHz on different occasions. This learning effect was only demonstrable during the first 0 5 min of each Békesy sweep recording, i e a hearing threshold difference between the up and down frequency sweep in the range of 0.25 to 0.5 kHz.

We also found that a relative underpressure in the middle ear cavity can affect the hearing threshold with both hearing loss and hearing gain for frequencies in the range of 0.5 to 6 kHz. It is known from earlier studies (Békésy, 1929, Arnold & Schindler, 1963; Truswell et al, 1979) that a relative overpressure in the external ear canal causes hearing loss. In the present study we simulated the true effect of pressure disequilibrium of the middle ear (Elner et al, 1971) on hearing sensitivity. A negative middle ear pressure is known to induce an increase in stiffness of both the tympanic membrane and of the round and oval windows (Ivarsson & Pedersen, 1977). This may explain the threshold gain at 2 and 6 kHz shown in the present investigation. The two frequencies correspond to the maxima of the positive reactance of the input impedance characteristic of the middle ear (Mehrgardt & Mellert, 1977), which can probably be reduced on increasing stiffness of the membranes. The temporary threshold shift caused by small middle ear pressure variation (< 5 cm H_2O), relative to the ambient pressure, includes 90 % of the shipyard-workers tested in this study This temporary threshold shift can be ignored by using the summed hearing thresholds between 2 and 8 kHz.

To reduce the effect of a temporary threshold shift caused by noise, our subjects were told to use hearing protectors the whole time during the preceding working-day and to have at least 16 hours' rest after noise exposure before a hearing test. Ward (1975) has reported that subjects with 24 hours of exposure to noise levels of 75 and 80 dB, which centered at 4 kHz, had completely recovered their hearing thresholds 16 hours after the exposure In this experiment, where the noise level was about 85 dB, the subjects required 24 hours for complete recovery.

The hearing threshold loss with advancing age in 'absence of damaging noise exposure" has been studied by several authors (e.g Hinchcliffe, 1959, Glorig & Nixon, 1962, Corso, 1963, Spoor, 1967, Burns & Robinson, 1970). In order to estimate the significance of the hearing threshold shifts caused by exposure to noise in the present study, each ear was corrected for presbyacusis according to the age-correction coefficients of Burns & Robinson (1970) for frequencies up to 6 kHz and according to the values of Hinchcliffe (1959) for higher frequencies.

Despite the use of hearing protectors about 50 % of the investigated workers at Kockums Shipyard had a significant noise-induced hearing threshold shift in the frequency range of 2 to 8 kHz within a period of 2 years, as tested by the above mentioned Békésy sweep method. The acoustic attenuation characteristics of new hearing protectors are evaluated in National Acoustic Laboratories on dummies and volontary subjects (e.g. Martin, 1977; Swedish Standard SS 882150, 1979). Earmuffs that had been used have been tested and the attenuation proved seriously reduced (Johansson, 1978). Else (1973) and Karmy & Coles (1975) have also pointed out that if the workers for some reason do not use the hearing protectors for even a small part of the working time, the protection is seriously reduced These observations may explain the greater hearing impairment of the "muff-men" than of the "plug-men" in the present study because it is much easier to remove earmuffs than earplugs.

We are of the opinion that a further increase of the reliability of the Békésy sweep hearing threshold measurements facilitates earlier detection of the hearing impairment caused by noise The present study showed that the reliability of measurements depends also on the application of the earphones, which has already been pointed out by Békésy (1947). An ear speculum adapted to the earphone for sound transmission to the external ear canal suggests an improvement of the reliability for one and the same ear in the frequency range of 2 to 8 kHz.

PRACTICAL IMPLICATIONS AND RECOMMENDATIONS

It appears from the present study that Békésy sweep audiometry with calculation of HT_{2-8} can have a wide use for industrial hearing conservation programs since its reliability has been proved to be better than that of manual pure tone audiometry This means that one can confirm a hearing impairment caused by noise at an earlier stage and thereby be able to:

1. Determine which working process/workshop that causes hearing impairment.
2. Estimate the effects of noise reduction programs at the actual working place
3. Estimate the efficiency of different kinds of personal hearing protectors.
4. Detect whether a new working process increases the risk of hearing damage.
5. Select noise-sensitive persons who tend to develop hearing impairment early

Békésy sweep audiometry showed that the workers using muffs had a greater hearing threshold shift within 2 years in the frequency range of 2 to 8 kHz than those using plugs. We think that this difference depends on the fact that muffs are easier to remove than plugs.

As long as the noise cannot be reduced at the source, i.e. the working places cannot be made less noisy, hearing must be protected more effectively than it is today. On the basis of the present investigations at Kockums Shipyard, the authors therefore recommend plugs at a moderate exposure to noise and plugs combined with muffs when the noise is extreme.

CHAPTER III. EXPERIMENTAL NOISE STUDIES IN ANIMALS

INTRODUCTION

The purpose of most programs concerning occupational hearing loss is to protect the hearing of the workers To achieve this two measures are used, reduction of noise and protection against noise This requires knowledge of the relation between exposure to noise and hearing impairment

One popular basis for present noise regulations is the "equal-energy principle", which postulates that permanent damage to hearing is proportionally related to the total sound energy (which is a product of sound intensity and duration) that has entered the ear, which means that equal noise energy causes equal hearing loss The equal-energy principle was originally proposed for comparisons of daily occupational exposure to noise allowing an increase of 3 dB in sound pressure level for each halving of the duration of continuous noise exposure (U S EPA, 1973) Furthermore, Atherley & Martin (1971) extended the equal-energy concept to include impulse noise

The equal-energy principle is supported by some investigations (Burns & Robinson, 1970, Martin, 1973) On the other hand, analysis of workers performed by Scheiblechner (1974) and Rop et al (1979) indicate that the equal-energy principle is not always valid Furthermore, observations of occupational noise containing impulses (Passchier-Vermeer, 1971, Bruel, 1976) seem to involve a higher risk of hearing loss than that indicated by the equal-energy rule

Prospective studies of the noise-damage relationship could be facilitated by repeated measurement of the noise exposure, as described in chapter I combined with measurement of the hearing threshold described in chapter II Simultaneous measurement of noise and hearing might then contribute to the understanding of the relation between noise exposure and hearing impairment

The main purpose of a prospective hearing conservation program is early detection of those workers who develop undue hearing losses and to take measures against noise to prevent progress of hearing impairment This means, however, that the noise environment is changed which makes an evaluation of the noise-damage relationship more difficult

The disadvantages of both retrospective and prospective investigations of the noise hearing damage relationship in man justifies experimental studies in animals Such studies were performed already in the beginning of the century (Wittmaack, 1907) Guild s (1921) description of a graphic reconstruc-

tion of the cochlea and Davis' (1976) development of electrophysiological measurement of hearing provided new possibilities for evaluating noise-induced damage to the inner ear

Most earlier studies were concerned more with the pathologic morphology and pathophysiology of hearing The surface preparation method of the inner ear, which was introduced by Neubert (1952) and refined by Engstrom et al (1966) to a practical technique, made it possible to examine all hair cells of the cochlea This technique could be used for relating noise exposure to damage even in man, and important contributions were made by Bredberg (1968), Hawkins & Johnsson (1975) and Johnsson (1979)

In animals the surface preparation technique is an excellent basis for quantitative studies of the effects of different kinds of noxious agents (Falk et al, 1973) The advantages of animal experiments for correlative studies between noise and damage to the inner ear and to hearing have been emphasized by Eldredge et al(1973), and important contributions to the understanding of the noise-damage relationship have been made by Ruedi & Furrer (1946), Stockwell et al (1969), Ward & Nelson (1971), Spoendlin (1976), Henderson & Hamernik (1978) and Ward et al (1979)

Earlier studies were performed in different animal species and often with the use of sound variables, which are not comparable to industrial noise It is therefore difficult to draw conclusions concerning validity of the equal-energy principle

The aim of this study was to perform studies of the relation between noise exposure and histological damage to the inner ear from the hypothesis that the equal-energy rule is not generally valid for all kinds of noise

XII. HAIR CELL DAMAGE IN GUINEA PIG DUE TO DIFFERENT KINDS OF NOISE

The aim of the animal experiments was to study the relation between exposure to noise and damage to the inner ear It was thus required that the variables of the noise should be thoroughly controlled, and that the sound levels should be comparable to occupational sound levels and durations and the exposures should be varied in agreement with the equal-energy concept

MATERIALS AND METHODS

Pigmented, female, dark-eyed guinea pigs from an old, half-inbred stock without known hereditary defects were used in order to avoid other known characters which could influence on the noise-induced hair cell damage (Stockwell, 1969, Ulehlová & Voldrich, 1978, Carter, 1980) Eighty guinea pigs were exposed in groups of five in order to minimize the inter-individual variation During the noise-exposures the animals were placed in a circular wire mesh cage, where they had free access to food and water The acoustics of each exposure was thoroughly controlled and recorded using digital memory equipment

The guinea pigs were divided into four exposure series In series I, groups of guinea pigs were exposed to 1 33 kHz continuous pure tone for 6 hours The groups were exposed to different intensities, which were varied in stepwise from 102 to 120 dB SPL Series II was performed like series I, but with 3 85 kHz continuous pure tone stimulus The animals of series III and IV were exposed to the impulse from an impulse sound generator described by Erlandsson et al (1980) which could repeat its characteristic impulse for more than 72 hours without significant change Analysis of the spectrum of the impulse revealed most of the energy to be located around 1 kHz By changing the rate of the hammer the equivalent sound level, L_{eq}, could be varied In series III groups of animals were exposed to the impulse noise for 6 hours at the L_{eq} 92 7, 97 7 and 102 0 dB In series IV, the different groups of guinea pigs were exposed to the same L_{eq} of 102 dB, but the duration was doubled for each group from 6 to 48 hours

All animals were killed four weeks after the exposures No signs of earlier or existing otitis media were found Both ears were prepared with the epon-embedding method (Wersall, 1956) One of the cochleae was cut in halves and the cochlear segments were mounted in epon for surface studies using a technique modified from Spoendlin & Brun (1974) Existing and absent hair

cells were counted in accordance with Engstrom et al (1966). To minimize differences in evaluation particularly of the more severe damaged areas (Stockwell, 1969), only one technician did all counting of the more damaged areas of the cochlea

Only damage to the outer hair cells (OHC) was taken into account in the present work The damage is described in terms of percentages of OHC-loss and the values in the different series are presented to show the distribution of the damage in the cochlea. To facilitate comparison between cochlear distance and the position of the stimulus frequency, Greenwood's (1961) function, derived from Békésy's (1960) elasticity data, was used.

The influence of aging was estimated from analysis of the OHC-losses in five non-exposed control animals With the exception of exposure to noise they were otherwise treated in the same manner and were of the same age as the experimental animals.

RESULTS AND DISCUSSION

The OHC-loss in the control animals was low, 0.58 ± 0.27 %, which is in agreement with the results of Federspil (1972) and Ůlehlová (1973) The OHC-loss in the control animals was significantly smaller than that in any of the noise exposed group of animals The excess of damaged OHC in all the exposed animals was therefore attributed to noise exposure

The damage produced by continuous pure-tone exposure was located in the cochleae corresponding to the stimulus frequencies. At increasing stimulus intensities, damage also appeared in the segments, which surrounded the segments of the stimulus frequencies The extension of the damage in the basal direction might be explained by the traveling wave hypothesis (Békésy, 1960, Tonndorf, 1979) whereas the damage in the next segment in the apical direction (Dallos, 1973) could be caused by subharmonic distortion components.

Total OHC-loss percentages in all the animals were calculated and plotted as mean values for each exposure group as a function of stimulus intensity (fig 16) In this figure both pure-tone series showed a threshold of intensity (critical level), where the mean damage started to increase. The existence of such critical levels has earlier been described by Spoendlin & Brun (1973) and Ward et al (1979)

For intensities below the critical level, the damage to the OHC does not appear to vary in proportion to variation of intensity in any of the pure-tone series (I-II) In the animals exposed to impulse noise (series III),

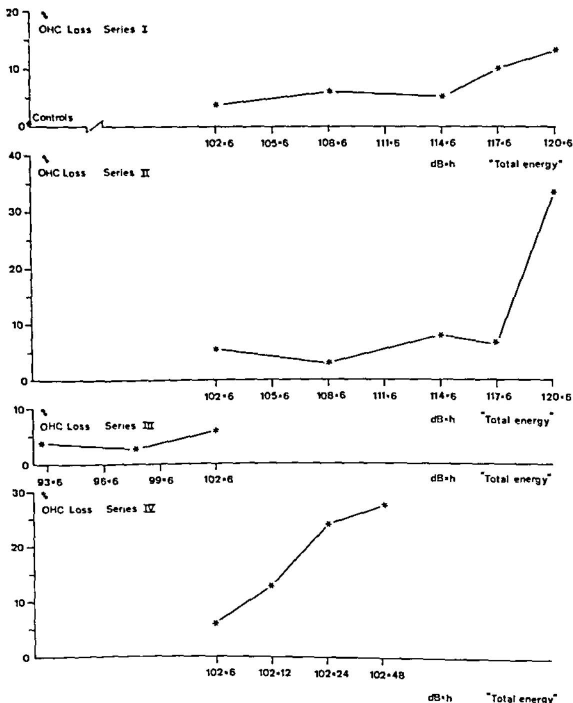


Fig 16. Mean values of total OHC-loss percentages for each group of noise-exposed guinea pigs plotted as function of the total sound energy.

there was also a lack of relationship between the values of the damage and variations of the total energy (fig 16) This might mean that below the critical level the hypothesis that the damage is proportional to the total noise energy is not valid for OHC-loss

The extent of the OHC-damage above the critical level in the animals exposed to pure-tones of 120 dB SPL was much worse in those exposed to 3 85 kHz pure-tone than in those exposed to 1 33 kHz A similar result was described by Stockwell et al (1969) It thus appears that in the guinea pig, a 3 85 kHz pure tone has a more damaging effect than a 1 33 kHz pure-tone at equal intensities above the critical level, i e the equal-energy rule is not valid under these circumstances

A different scheme of exposures was performed in the IVth series All animal groups were exposed to the same sound level but the total energy was increased between the groups by doubling the duration of exposure The resulting damage showed a relation between OHC-damage and the total energy, which seemed to be valid from the start up to 24 hours, after which an asymptotic approach to a maximum damage is indicated (fig 16) The described pattern is supported by the results of Spoendlin & Brun (1973)

Comparison between the damage values in the group of animals in series III exposed to impulse noise at 102 dB L_{eq}, 6 h, and the damage in the group of animals in series I exposed to pure-tone 1 33 kHz revealed slightly greater damage in the animals exposed to impulse noise (p 0 11) This finding might indicate a more damaging effect of the impulse noise than the corresponding pure-tone and consequently argues against the equal-energy concept

omparison of the damage in the animals exposed to impulse noise (series IV) nd the damage in the animals exposed to pure-tone demonstrates worse damage t the corresponding total energy (fig 16) The damage in the impulse noise xposed animals of series IV also exceeds the damage in the animals exposed o pure-tone 1 33 kHz 6h at 120 dB SPL, which is a strong argument against he application of the total energy rule to this situation

ONCLUSION

This investigation demonstrated the existence of a critical level There are indications for different mechanisms for the damage below and above this level and that these mechanisms are not possible to combine according to the total energy concept However, when the total energy was varied by change of the exposure time the damage to the OHC did vary with the total sound

energy. This reans that there is evidence both in favour of, and against, the equal-energy concept and that a generalized equal-energy or total energy principle cannot be applied throughout the whole range of the different parameters of noise concerning histological damage

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A different scheme of exposures was performed in the IVth series All animal groups were exposed to the same sound level but the total energy was increased between the groups by doubling the duration of exposure The resulting damage showed a relation between OHC-damage and the total energy, which seemed to be valid from the start up to 24 hours, after which an asymptotic approach to a maximum damage is indicated (fig 16) The described pattern is supported by the results of Spoendlin & Brun (1973)

Comparison between the damage values in the group of animals in series III exposed to impulse noise at 102 dB L_{eq}, 6 h, and the damage in the group of animals in series I exposed to pure-tone 1 33 kHz revealed slightly greater damage in the animals exposed to impulse noise (p 0 11) This finding might indicate a more damaging effect of the impulse noise than the corresponding pure-tone and consequently argues against the equal-energy concept

omparison of the damage in the animals exposed to impulse noise (series IV) nd the damage in the animals exposed to pure-tone demonstrates worse damage t the corresponding total energy (fig 16) The damage in the impulse noise xposed animals of series IV also exceeds the damage in the animals exposed o pure-tone 1 33 kHz 6h at 120 dB SPL, which is a strong argument against he application of the total energy rule to this situation

ONCLUSION

This investigation demonstrated the existence of a critical level There are indications for different mechanisms for the damage below and above this level and that these mechanisms are not possible to combine according to the total energy concept However, when the total energy was varied by change of the exposure time the damage to the OHC did vary with the total sound

energy. This means that there is evidence both in favour of, and against, the equal-energy concept and that a generalized equal-energy or total energy principle cannot be applied throughout the whole range of the different parameters of noise concerning histological damage.

CHAPTER IV. CONCLUDING REMARKS

The concept of the equal-energy principle, which has been discussed earlier in this investigation has recieved a great deal of support by several authors. Many arguments, however, contradict the equal-energy concept. The experimental noise studies in animals in this investigation were performed in order to elucidate the relationships between the variables of noise and impairment of hearing. In the interpretation of the validity of the conclusions in paper XII, the relation between hearing and loss of outer hair cells (OHC) as well as the relation between animal experiments and human hearing loss deserves commentation.

OHC are the first cells to be damaged by noise (Bohne, 1976). The exact role of the OHC-system in hearing is not known. Stebbins et al (1979) indicate that loss of OHC was most often correlated with hearing threshold shifts of 50 dB or less, whereas loss of inner hair cells (IHC) was observed at threshold shifts exceeding 50 dB. Earlier reports of lack of consistency between audiometric and histologic findings reviewed by Lipscomb et al (1977) seem to be due to discrepancies in techniques and species of animals used. Beyond doubt the OHC participate in the hearing process and there are indications that OHC serve as current pumps of receptor potentials providing a sensitizing influence to the IHC (Dallos & Cheatham, 1976, Dallos & Harris, 1978).

Findings in animal experiments are by no means simple to transform into conclusions valid for man. Only a few comparative data are available. Ruedi (1954) was thus able to compare man and guinea pig and found the latter to be more vulnerable. By means of defining the transition from physiological fatigue to pathological hearing by narrowing of the difference limen in Békésy recordings in man Ruedi was able to find the critical level. Eldredge et al (1973) also support the assertations of similarities between man and chinchilla concerning the critical level of damaging sound intensity, frequency spectrum hazard and recovery from temporary threshold shifts after exposure to noise and they also assume the pathological morphology and physiology in chinchilla to be valid also in man.

It thus seems reasonable to believe that the fundamental mechanisms of noise damage described in animals have their equivalents in the human ear and hearing and that the conclusions of restricted validity of the equal-energy concept should also apply to man. The pure-tone exposures do not, of course, correspond to the spectra of industrial noise, but the conclusions drawn from these exposures are supported by results of wide band noise exposure.

(Spoendlin, 1976, Ward et al, 1979) The series of continuous noise exposure, extended up to 48 hours, which more clearly reflects the non-validity of the equal-energy concept, is not representative of occupational noise. It might, however, indicate that prolonged occupational or non-occupational noise exposure may contribute to a more injurious effect (Johson & Farin, 1977). Of great importance are also the observations by Passchier-Vermeer (1973) and Ward et al (1979) that intermittent distribution of the noise is less harmful than what is indicated by the equal energy concept

The risk of hearing loss as given by ISO R1999, is considered only for the arithmetic average of the permanent threshold shift for 500, 1000 and 2000 Hz exceeding 25 dB

From numerous investigations it is evident that the noise sensitive range is situated between 2 and 8 kHz and much work has been devoted to the 4 kHz-dip The consequences of the valid recommendations might be elucidated best by the example given in paper VIII and IX In the subjects involved in these investigations very little or no progress in hearing loss was found in the 0 5-2 kHz range, but 36 % of the investigated ears may have developed a progressive hearing loss in the 2-8 kHz range Corresponding ideas of the insufficiency of the ISO R1999 are given by Kryter (1973), Passchier-Vermeer (1974) and Welleschik & Raber (1978) and it seems that other measures for early detection of noise-susceptible individuals are urgent (Humes, 1977; Michael & Bienvenue, 1978)

CONCLUSIONS

The valid criteria for the assessment of occupational noise for the purpose of hearing conservation appear to be insufficient for evaluation of many of the prevailing occupational noise environments The present investigations have shown that occupational noise, including impulse noise, should be measured at the entrance to the ear canal for correct interpretation of the spectrum, and it is also shown that an ear borne noise dosimeter, the construction and application of which is presented, fills this purpose. It is also shown that Békesy sweep audiometry, correctly performed by means of some special procedures, in the measurement of hearing and calculation of the results gives a very high degree of accuracy, which enables very early detection of progressive hearing loss In a series of animal experiments it is shown that in some respects the equal-energy concept is not valid.

Taken together, the results indicate the need for new guidelines for assessment of occupational noise and evaluation of the risk of noise-induced hearing impairment

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CALCULATION OF NOISE DOSE FROM TIME DISTRIBUTION OF SOUND LEVELS

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Key Words. Noise dose, L_{eq}, Class interval.

Abstract. The noise from ten different sites in a machine factory was measured and cumulative time distributions of the sound pressure were constructed. From these distributions it is possible to calculate the energy mean level, the equivalent sound level as well as the average sound pressure level. Calculations based on knowledge of the times during which only a few sound pressure levels are exceeded give values deviating systematically from the exact value. Because of the systematic nature of the deviations corrections can be made for them. The accuracy of this method is relatively high.

INTRODUCTION

Sound as it is received by the human ear is small alternating positive and negative alterations of the prevailing ambient air pressure The range of such variation which can be translated to auditory perception is generally very large and it is therefore convenient to measure them by means of a logarithmic scale, the dB-scale As these pressure variations are vary rapid, sound measurements must be expressed as a mean value over a certain time. The most common way to obtain this value is to integrate the squared function of the sound pressure over time, which is proportional to the sound energy. Averaged over the time it may be expressed as the "energy mean level", the "energy equivalent level", or the "equivalent sound level" The equivalent sound level is a concept which has been widely used in predicting annoyance and risk of damage by various types of noise Recently investigations have been published which imply that phenomena caused by noise may be better determined if they are compared to the average sound pressure level than to the equivalent sound level (Pfander, 1975, Ward, 1977)

The aim of this investigation was to investigate the accuracy of certain methods by means of which the energy mean level and the equivalent sound level are calculated from measurements of the total time certain sound pressure levels have been exceeded

MATERIALS AND METHODS

The noise from different working places in a machine factory was measured with a Bruel & Kjaer 2206 precision sound level meter and recorded on a Nagra tape recorder The tapes were then analysed and a cumulative time distribution of the sound pressure was calculated and presented in a diagram.*
A diagram can be constructed by summing up the time certain sound pressure levels have been exceeded, fig 1a. Connected values of summed time and sound pressure can then be represented in a new pressure versus time diagram, fig.1b. The area enclosed by the curve and the axes in fig. 1b is equivalent to the total area of the positive peaks in fig. 1a and has the dimension Pa x s. From this area it is possible to calculate the average sound pressure level.

* These measurements have kindly been placed at our disposal by J. Svensson, Ingemansson Acoustics, Gothenburg.

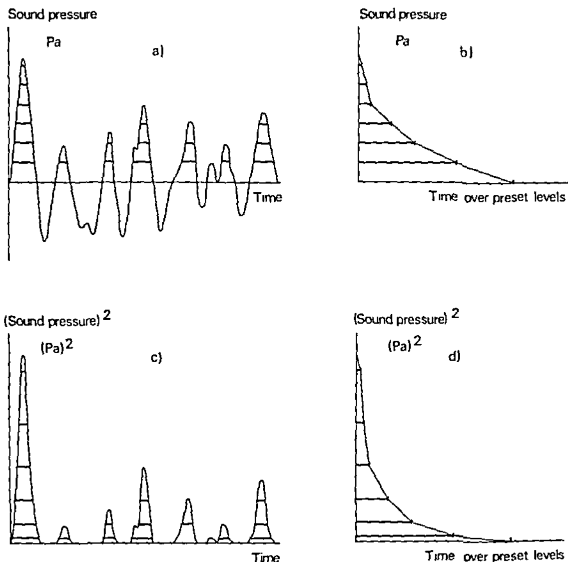


Fig. 1. Sound pressure and sound energy distributions and their cumulative representations.

Another way of presenting the noise is to calculate or measure the total sound energy E, that passes through the unit area in time T, assuming plane wave radiation

$$E = \int_0^T \frac{p^2}{\rho_0 \times C} \, dt \qquad (1)$$

where p is the instantaneous sound pressure amplitude, $\rho_0 \times C$ is the characteristic impedance and T is the total measuring time The impedance can be treated as a constant and the sound energy is therefore proportional to the squared sound pressure. The squared positive sound pressure amplitudes of fig. 1a are given in fig. 1c By summing up the times certain energy levels have been exceeded a similar diagram as 1b can be constructed (fig. 1d) but

the enclosed area now represents something which is proportional to the total sound energy and has the dimension $Pa^2 \times s$ From this area it is possible to calculate the energy mean level and the equivalent sound level.

It is also obvious that by squaring the pressure level values fig. 1b can be transformed into fig. 1d without changing the time-values It is therefore possible to calculate both the average sound pressure level and the energy mean level from measurements of the total time certain sound pressure levels have been exceeded during an experiment

The cumulative time analysis could be made employing different numbers of pressure or energy levels Owing to the form of the distribution, which from a high pressure-energy value rapidly drops off with time ending in a long tail, these distributions are best presented in a log-log diagram In fig 2 is shown a distribution where the cumulative analysis is performed in 1 dB(A) steps. This means that the time duration has to be determined many times but in return the area and thus the average sound pressure or the energy mean level can be determined with a high accuracy

As seen from the figure it should also be possible to get a rather good area determination if one connected the time values for each fifth or tenth dB(A). These lines can be expressed by the equation

$$\log t = a - b \times \log (p^2) \qquad (2)$$

which may be evaluated to an expression which is proportional to the sound energy

$$(p^2) = \frac{10^{a/b}}{t^{1/b}} \qquad (3)$$

This may be integrated giving the total energy between the two points T_1 and T_2

$$E = \int_{T_1}^{T_2} (p)^2 dt = \int_{T_1}^{T_2} \frac{10^{\alpha}}{t^{\beta}} dt = \frac{10^{\alpha}}{1-\beta} \left[T_2^{(1-\beta)} - T_1^{(1-\beta)} \right] \qquad (4)$$

This formula may be used for time intervals $(T_2 - T_1)$ of different length (fig 2)

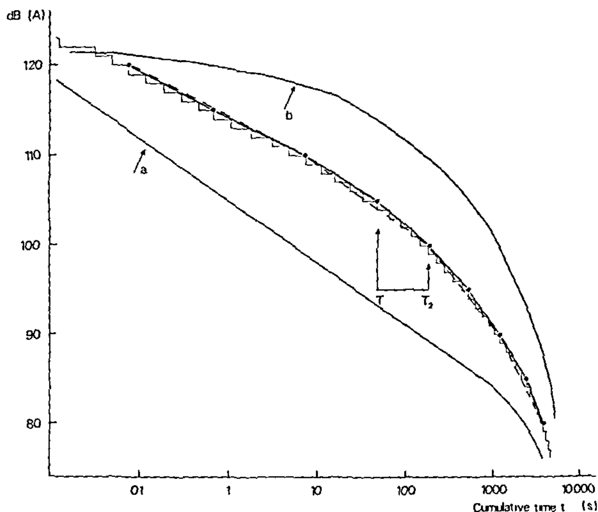


Fig 2 Examples of different cumulative distributions obtained in analysing noise from a machine factory a) high content of impulse noise b) low content of impulse noise

RESULTS AND DISCUSSION

The noise measurements were performed at a machine factory and as noise sources different types of machines have served Some of the machines produced a noise with contributions of impulse noise and some produced a more stable noise

The tape-recordings of 10 different noise sources were analysed in the way presented above and cumulative time distributions of the sound pressure levels were derived These distributions are of the same form as the one shown in fig 2 A distribution w th a large contribution of impulse noise is represented by a straighter curve a) and a distribution with a low content of impulse noise is represented by a curve like (b) in fig 2 For these 10 noise sources we have calculated the energy mean level and the equivalent sound level, L_{eq}, for various configurations of sound levels The L_{eq}-value which is given

by

$$L_{eq} = 10 \log \frac{1}{T} \int_0^T p^2 \, dt \tag{5}$$

can be derived from equation (4) which conforms to the formula for calculating the L_{eq}-value according to ISO R1996 T is the total time

$$L_{eq} = 10 \log \left[\frac{1}{100} \sum f_i \times 10^{L_i/10} \right] \tag{6}$$

Where L_i is the sound level in dB(A) corresponding to the class-midpoint of the class i For class intervals not greater than 5 dB(A) the arithmetic means can be used and for larger intervals logarithmic averaging should be used The time-interval f_i is expressed as a percentage of the relevant time (or total time) The L_{eq}-value was calculated for various class intervals and are presented in table I The values given in columns 2, 3 and 4 are calculated by means of equation (4) whereas the values in column 5 were calculated from the arithmetic mean of the class-limits according to ISO R1996

With such logarithmic intervals as those mentioned above the energy contributions to the total energy from intervals below 75 dB(A) are very small (< 1%) and have therefore been neglected For the same reasons it is also of great importance to include the highest sound pressure levels even if the times are very short and also here we have tried to incorporate energy contributions down to about 2-3 % of the total energy

For practical reasons the smallest interval was 2 dB(A) and the L_{eq}-values obtained with this interval are considered to be the most accurate (Column 2) We also used intervals of 5 and 10 dB(A) (columns 3 and 4) and the differences between column 2 and these are given in columns 7 and 8 The differences are very small with mean values 0 2 and 0 9 dB(A) and all with the same sign We are here most probably dealing with a systematic error depending on the fact that the distributions are curved in the same direction between the measuring points (fig 2) The differences between the values in column 2 and those calculated according to ISO R1996 are shown in column 9 and are of both signs.

We also tried to use as few levels as possible in calculating the L_{eq}-value to see what consequences it has Using only 3 levels 85 dB(A), 105 dB(A) and the peak value the L_{eq}-value is systematically underestimated (columns 6 and 10) The mean value of the differences is 2 6 dB(A) Owing to the systematic nature of these differences it should be possible to get a rather good L_{eq}-value if the calculated value was corrected with a certain factor. The correction factors and the estimated errors are: +0 2 and ± 0.2 dB(A) for

Table I *Calculated L_{eq}-values in dB(A) for different class intervals*

Site	L_{eq} according to Equation (4)			ISO R1996	peak 105 dB(A) 85 dB(A)	L_{eq} differences			
No Col 1	2 dB(A) Col 2	5 dB(A) Col 3	10 dB(A) Col 4	5 dB(A) Col 5	Col 6	Col 2 - 3 dB(A) Col 7	Col 2 - 4 dB(A) Col 8	Col 2 - 5 dB(A) Col 9	Col 2 - 6 dB(A) Col 10
1	104 9	104 5	103 7	104 2	102 5	0 4	1 2	0 7	2 4
2	104 8	104 5	103 2	105 0	102 3	0 3	1 6	-0 2	2 5
3	99 2	98 7	98 4	98 7	96 3	0 5	0 8	0 5	2 9
4	91 6	91 3	90 7	91 8	88 5	0 3	0 9	-0 2	3 1
5	90 8	90 6	90 2	91 2	88 0	0 2	0 6	-0 4	2 8
6	88 3	88 1	87 1	88 7	84 5	0 2	1 2	-0 4	3 8
7	90 4	90 4	89 7	90 6	86 3	0	0 7	-0 2	4 1
8	94 1	94 0	93 7	93 8	92 5	0 1	0 4	0 3	1 6
9	92 4	92 3	92 2	92 6	91 3	0 1	0 2	-0 2	1 1
10	85 4	85 3	84 5	85 8	83 3	0 1	0 9	-0 4	2 1
Mean value						0 2	0 9	-0 1	2 6

col. 3, +0.9 and ± 0.5 dB(A) for col 4, +2 6 and ± 1 0 dB(A) for col. 6, and no correction and ± 0.5 dB(A) for col. 5

In the methodological description only the positive variations of the sound pressure were treated but investigations indicate that the difference in energy content between the positive and negative alterations are small.

In these calculations no consideration was given to experimental errors which in these measurements can appear as errors in determining the sound pressure level and the accumulated time. Such errors add very little to the errors given for the correction factors and the estimated errors

CONCLUSIONS

We have derived a method by means of which it is possible to calculate the energy mean level, the L_{eq}-value and also the mean sound pressure level for dB-class intervals which are 5 dB(A) or greater and is practically applicable even if only 2 levels and the peak value are known from the time distribution of the sound pressure The precision which can be obtained with this method makes it possible to use ear-borne noise dosimeters incorporating only few preset sound levels (Erlandsson, 1976)

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AN IMPULSE SOUND GENERATOR AND SOME COMPARATIVE EXPERIMENTS WITH DIFFERENT NOISE DOSIMETERS

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Key Words Impulse sound generator, Reproducibility of impulse sound, Impulse sound parameters, Noise dosimeters, Ear-borne sound level duration meter

Abstract An impulse sound generator has been constructed which gives the same sound pressure wave pattern during over 10^5 impacts with very small variations This device has been used in evaluating the response of different types of noise dosimeters for impulse sounds

INTRODUCTION

The industrial noise contains elements of impulse noise, whose peak pressures and impact rates may vary within broad limits. The risks of hearing impairment due to impulse noise have recently been emphasized by Bruel (1976) and during the last 20 years several experiments have been performed where the effect of impulse noise on both human and animal hearing has been investigated.

In the laboratory several methods have been used to create impulse sounds, either through mechanical or electrical methods. Ward et al (1961) used a mechanical toy cricket where a concave steel sheet snaps back and forth. They also discharged a high-capacity condenser through a thyratron in series with a speaker and an exponential horn. The same device with slight modifications was used by McRobert & Ward (1973). The capacitive spark discharge has also been used by Hamernik et al (1974). The authors previously mentioned have described the impulse sound in terms of peak sound pressure, repetition rate and equivalent continuous noise level but have neither shown the sound pressure pattern nor mentioned anything about the reproducibility of the impulse sound pattern or its energy content. Martin & Rood (1974) who have used four hammers striking a metal plate in rapid succession, have presented several noise parameters and the impacts seem to be well standardized.

The reason why we have constructed this generator is to obtain a reliable tool with which impulse noise can be produced and with which different methods of measuring the physical parameters of this noise can be investigated. This impulse sound generator can be used for exposing animals and for testing noise dosimeters.

EXPERIMENTAL ARRANGEMENTS

To fulfil the demands for an impulse sound generator where it is possible to vary the hight of the pressure peak, the duration of the total pulse, the main frequency content and the repetition rates of the impulses, we have constructed a motor driven hammer. The hammer is shown in fig 1 On the shaft of the motor there is a small rod which interacts with the shaft of the hammer When the motor is turning, the hammer is elevated and then falls on the plate The rotating speed is generally 75 rpm but can be changed. The hammer-head is filled with lead to avoid rebounding To get the same sound pressure patterns it is important that the hammer-head falls flat on the plate. The noise from the motor and from other parts of the hammer is about 70 dB re. 2.10^{-5} Pa and thus unimportant compared to the hammer blows.

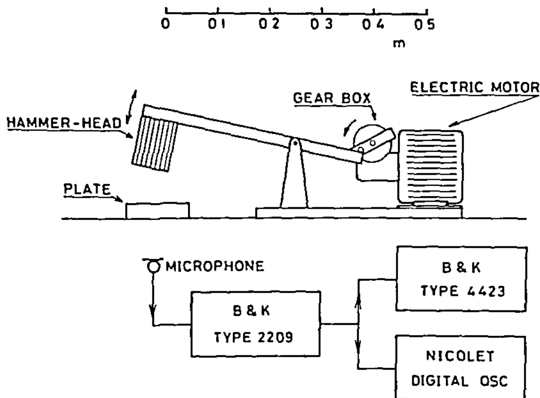


Fig. 1. The impulse sound generator and block diagram of experimental set-up.

The hammer tests and the following experiments were performed in a non-reveberent room which is roughly 4.25 x 2 25 x 2.15 m. To register the impulse sound a Bruel & Kjaer impulse precision sound level meter type B&K 2209 was used with an A-filter and equipped with a 1/2" microphone B&K 4165. The microphone was placed 25 cm under the generator. The amplified signal was then fed to a Nicolet 1090A digital oscilloscope and recorded in a 4096 word (12 bits/word) memory. The whole system was calibrated with a B&K 4220 pistonphone with a frequency of 250 Hz and a sound level of 123.9 ($\pm$ 0.2) dB $_{eff}$re 2 10^{-5} Pa. An acoustical calibrator B&K 4230 gave a calibration point at a lower sound level 93.8 ($\pm$ 0.3) dB $_{eff}$ re 2.10^{-5} Pa and with a frequency of 1000 Hz but with lower accuracy than the pistonphone. To control the reliability and reproducibility of the impulse sound generator the sound level meter was also coupled to a B&K 4423 noise dosimeter. At regular intervalls the dosimeter was read.

In another experiment the impulse sound generator has been used to control the ability to register impulse sound of 3 different types of noise dosimeters, B&K 4423, B&K 4424 and Crafon SLD. The B&K 4423 was used with time constant 1 ms and with the sound level meter B&K 2209 set at such a range that no overload time was registered. The B&K 4424 is a pocketsize dosimeter equipped with a 1/2" microphone and with a time constant of 500 ms. The Crafon SLD is an ear-borne sound level duration meter which utilizes photographic film as the storage element. It measures the time during which the sound pressure has exceeded two preset levels (Erlandsson et al, 1976a; Erlandsson et al, 1976b). In this test two meters with the preset levels at 85, 95, 105 and 115 dB(A) were used for calculating the L_{eq}-value. The microphones are very small (1.2 mm diameter) and they were arranged close around the microphone of the B&K 2209 together with the B&K 4424 microphone. All fronts of the microphones were placed in the same plane and perpendicular to the direction from the hammer. The sound pressure pattern was controlled to be the same within the whole area occupied by the microphones. The B&K dosimeters were calibrated with the pistonphone according to standard procedures. The B&K 2209 was also connected to the Nicolet digital oscilloscope. In this way the vertical axis to the oscilloscope was calibrated directly in terms of sound pressure units Pa. By squaring and integrating the sound pressure over the time we obtained a number which is proportional to the energy content (Pa^2.s). From this value, L_{eq} can be calculated if we know the repetition rate of the impulse sound generator.

RESULTS AND DISCUSSIONS

Reproducibility of impulse sound generator

The impulse from a hammer blow was recorded with the digital oscilloscope on each occasion In fig. 2 two impulse configurations are shown, the first at the start and the second 50.000 hammer blows later. There is no difference in the pressure pattern during the first 7 ms where about 85 % of the energy content is located Only in the later oscillations are there some differences It is possible to operate the hammer for more than 72 hours continuously without greater changes than 0.1 dB(A) in the L_{eq}-value, read from the B&K 4423.

Many authors have tried to pick out certain parameters from a sound impulse which may be of importance We have measured some of these at various times during the experiment and listed them in table I In columns 2, 3 and 6 the noise doses are listed as read from the B&K 4423 in arbitrary units, the calculated "energy content" from the oscilloscope graph in Pa^2 s during the

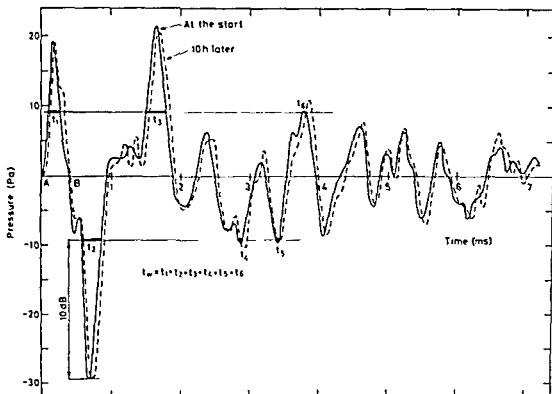


Fig. 2. Sound pressure (A-filtered) versus time for two impacts; solid curve at the start and interrupted curve 10 hours later.

Table I *Impulse noise parameters (A-filtered) for the impulse sound generator, at different times during a test run.*

Test time (h)	B&K 4423 arb units	Calculat. "sound energy" ($Pa^2.s$)	Effective duration (ms)	A-duration (ms)	Peak sound pressure (Pa)
0	111	0.409	0.94	0.47	28.1
1 5	111	0.412	0.97	0.43	28.2
3	112	0.418	0.92	0.42	28.8
4 5	113	0.412	0.95	0.41	28.5
8.5	114	0.421	0.99	0.41	28.4
10 0	115	0.421	0.97	0.42	28.8

first 7 ms and the maximum peak sound pressure in Pa. The differences between the values at the different times are small (about 2 %). In column 4 the effective duration (t_W) is given according to Pfander (1975). This is the total time the pressure wave exceeds a pressure value (both positive and negative) which is 10 dB(A) lower than the absolute maximum peak pressure, see fig. 2. In column 5 we have calculated the pressure wave duration (A-duration) according to Coles et al (1968). The A-duration is the time required for the initial or principal pressure wave to rise to its positive peak and return momentarily to ambient from A to B in fig. 2. The differences between the experimental values for these time definitions are small, which means that the impulse sound is the same during the entire experiment

Response of different noise dosimeters for impulse noise

The resulting L_{eq}-values of the experiments with the different dosimeters are presented in table II. Three different experimental arrangements each with only minor alterations have been used. Three different B&K 4424 and 5 pairs of Crafon SLD:s were used. The agreement between the calculated oscilloscope graph values, the Crafon SLD values and the B&K 4423 values are good with no significant trend within the different test runs.

Table II. *Comparison between the L_{eq}-values calculated from the oscilloscope graph and recorded by different noise dosimeters during test runs with the impulse sound generator.*

Equivalent continuous sound level L_{eq} dB (A)			
Test run	1	2	3
Oscilloscope graph	92.4	91.9	90.0
Crafon SLD	92.0	93.0	88.6
B&K 4423	91.3	92.0	90.0
B&K 4424	81.8	82.6	82.2

The repetition rate of the hammer was 1.25 impacts/s and the peak sound pressure 28-30 Pa (A) (123-123.5 dB(A)). If Martin's equation (Martin, 1970) is used to calculate the L_{eq}-value in these experiments, a value is obtained which is 2 dB(A) lower than the value which we calculated from the oscilloscope graph This is contrary to what Martin & Rood (1974) found. In their impact experiment they found a higher value calculated from the equation than from the B&K 4423 Our value which is lower may be explained by the difficulties in evaluating the t_e time in this experiment. The t_e is the time taken by the waveform envelope to decay to 1/e of the initial peak sound pressure (Martin, 1970).

There is a big difference between the L_{eq}-values of the B&K 4423 and B&K 4424. At a repetion rate of 1.25 impacts/s the difference is about 9 dB(A). This is not in agreement with the differences obtained by Martin & Rood (1974) which are much smaller. In fig. 3, Martin & Rood's and our L_{eq}- differences between B&K 4423 and B&K 4424 are shown. We have also tested this difference at a repetition rate of 2.5 impacts/s. As the peak sound pressures are almost the same, 123-125 dB(A) in all the experiments and the impact sounds are all of the same nature the results are comparable. It is quite obvious that few impacts/s give a lower value on the B&K 4424 than on the B&K 4423 and a higher value when the impact rate is over 6. At about 5-6 impacts/s, there is an agreement between the two meters and the true L_{eq}-value as represented by the value calculated from the oscilloscope graph value This may be explained by the construction of the B&K 4424 which has a time constant of 500 ms and a lower intensity limit of 80 dB (A).

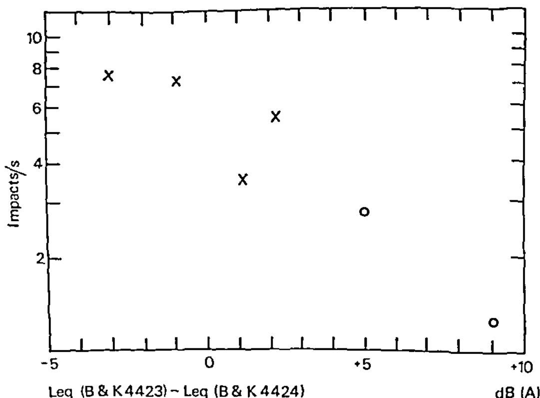


Fig. 3 Impacts/s of the impulse sound generator versus L_{eq} difference between B&K 4423 and B&K 4424; o=our values, x=experimental values from Martin & Rood (1974).

The Crafon SLD does not show this impact rate dependence. The photodiodes and the photographic film register with small errors every time interval the sound pressure has exceeded the preset value. The errors of the times registered on the film are less than 10 %. Another uncertainty can be introduced when the distribution of the individual pressure pulses between two limits is not arbitrary. These may give a total error not greater than ± 2 dB(A) in the L_{eq}-value. The calculated graph values, the Crafon SLD values and the B&K 4423 values are in good agreement and all of them are within 2 dB(A) at the different tests.

CONCLUSIONS

The impulse sound parameters may be defined as peak sound pressure, decay time, effective duration or A-duration. To define one or more of these parameters and keep them constant throughout an experiment is a rather crude way to say that the impulse sound has been constant. A more exact way to describe the impulse sound is to keep the sound pressure waveform pattern and thus the sound energy constant. Therefore we have constructed this impulse sound generator, which meets these requirements.

The other part of the experiment shows the results obtained by different dosimeters under this certain condition. Impulse sounds with repetition rates higher than 5 impacts/s are over-estimated and those with repetition rates of 3 or lower are under-estimated by one of the investigated dosimeters.

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NOISE DOSE MEASUREMENTS WITH STATIONARY AND EAR-BORNE MICROPHONES

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Key Words. Mobile microphone, Stationary microphone, Noise dose differences, Occupational noise.

Abstract. Precision measurement of noise doses with stationary and mobile, ear-borne, microphones were made on ten workers (emery-grinders, welders and platers). The workers selected in each group differed from one another in the amount they moved about during their working day. The differences in the reverberant field at their working places were also taken into account. Very large noise dose differences, up to 20 dB, between stationary and mobile dose measurements were found and it was not possible to find any correction factors for these differences.

INTRODUCTION

Industrial noise has been studied for many years In surveys of industrial noise levels, the noise has been measured mostly with stationary equipments (Friberg et al, 1958, Burns & Robinson, 1970), whereas some studies of the effect of occupational noise on hearing has been measured with a microphone mounted in the vicinity of the workers ear (Holmgren et al, 1971)

In most industries impulse noise is common and can cause errors in the reading of sound level meters depending on the response time of the meter (Hemingway & Christensen,1973, Wilkerson, 1974, Rosenheck & Hofmann, 1977) Also measuring of impulse noise correctly with older types of dosimeters offers problems, but the latest generation is much better in this respect (Svensson, 1978, Erlandsson et al, 1980b)

In the last years many investigations have been focused on the influence of the position of the microphone on the measurements (Muldoon, 1973, Olsen & Carhart, 1975, Kuhn & Burnett, 1977)

In the present investigation we have compared the influence of the position of stationary and mobile (ear-borne) microphones on the dose measured at different working places of men in different occupations in order to find out whether the differences are constant with regard to occupation, reverberant field at the working place or the degree of mobility of the worker We have also compared our results with earlier measurements made in the same workshop but with other types of equipment (Oxelbeck & Elmegren, 1973, Erlandsson et al, 1980a) in order to check whether the noise had changed in the meantime

EQUIPMENT AND METHODS

In our investigation of the noise at Kockums Shipyard, Malmo, Sweden, we used mostly the set-up shown in fig 1 Two identical parallel noise measuring channels were used, one for the stationary and one for the mobile microphone The microphones, 1/4 , Bruel & Kjaer (B & K) 4135, were connected to two precision sound level meters B & K 2209 The stationary microphone was placed as close to the worker as allowed by his activities The microphone of the mobile unit was placed near the entrance of the worker's ear canal (fig 2) The output signals from the sound level meters were fed to a two-track tape recorder, Kudelski, Nagra IV SJ In parallel with the signal to the tape recorder we had the possibility to connect both a dosimeter B & K 4423 and a digital memory oscilloscope, Nicolet 1090 A The registered signal of the oscilloscope could be recorded on a digital tape recorder, Nicolet 193

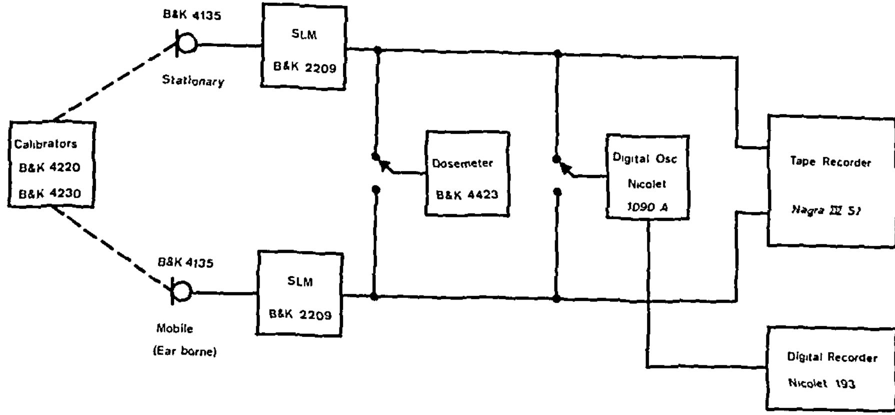


Fig. 1. Schematic drawing of the experimental equipment.



Fig 2 The position of the ear-borne microphone

The noise recorded was analysed at our laboratory with a frequency spectrometer B & K 2113 in conjunction with the dosimeter B & K 4423 or with a Fast Fourier Transform analyser Nicolet 444 A, and the results were presented on a digital plotter Tektronix 4662

Before any signals were recorded on the tape recorder, the latter was examined with outmost care The dynamic range was checked in order to avoid amplitudes of the input signals falling in the non-linear range of the recorder Also the frequency response of the recorder was measured and found to be better than $\pm$ 2 dB from 50 Hz to 25 kHz when the recording speed 38 cm/s was used.

The measurements were made in the following way. First the microphones were exposed to a calibration signal from a pistonphone, B & K 4220 for a well

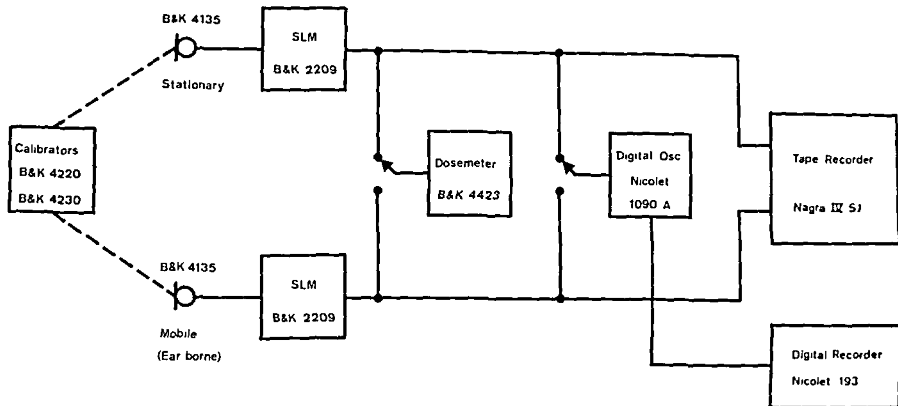


Fig. 1. Schematic drawing of the experimental equipment.

Ten measurements were performed in the boiler shop (fig 3) The double lines indicate baffles and the dotted lines work-tables or work-areas The walls in the workshop are made on concrete or brick and the height of the shop is about 10 meters in the left part and about 3 meters in the right part

Table I gives a short description of the different working positions When we chose the positions of the different measurements we selected workers differing from each other in occupation, in the amount they moved about and in the reverberant field at their working place

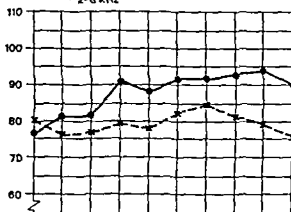
Table I [illegible]

Position	Occupation	Relative mobility of the worker	Comments
1	emery-grinder	high	low reverberation
2	emery-grinder	medium	low reverberation
3	emery-grinder	low	reverberation
4	welder	high	low reverberation
5	welder	medium	very low reverberation
6	welder	low	very high reverberation
7	welder	low	reverberation
8	plater	medium	high reverberation
9	plater	low	very low reverberation, punchpress
10	plater	low	very low reverberation

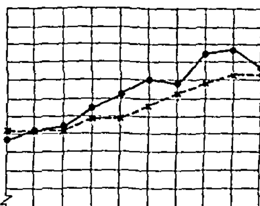
RESULTS AND DISCUSSION

The result of the octave band dose analysis of the tape recordings is shown in figs 4 and 5, and in table II the values of L_{eq} (A) and L_{eq} SPL are shown for respectively mobile and stationary measurements Figure 6 gives some results of corresponding Fast Fourier Transforms analyses, mean values of 512 samples As can be seen from the figures and table, there can be very large differences between the results of stationary and mobile (ear-borne) dosimetry The differences are not constant for a given occupation, a given mobility of the worker or for positions with the similar reverberant field

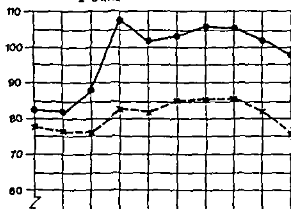
No 1 Emery-Grinder
Δ $Leq_{2-8 kHz}$ = 10.7 dB



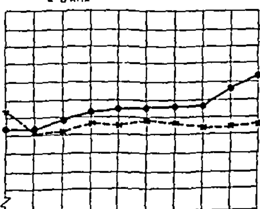
No 2 Emery-Grinder
Δ $Leq_{2-8 kHz}$ = 6.2 dB



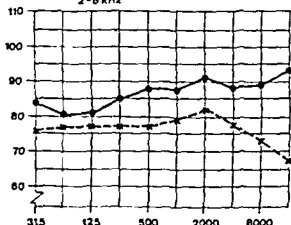
No 3 Emery-Grinder
Δ $Leq_{2-8 kHz}$ = 19.6 dB



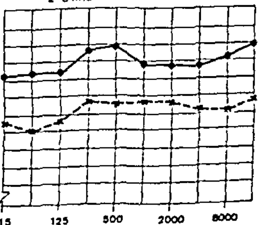
No 4 Welder
Δ $Leq_{2-8 kHz}$ = 7.7 dB



No 5 Welder
Δ $Leq_{2-8 kHz}$ = 12.0 dB



No 6 Welder
Δ $Leq_{2-8 kHz}$ = 12.9 dB



Leq in dB re 2 10^{-5} Pa

Octave frequency in Hz

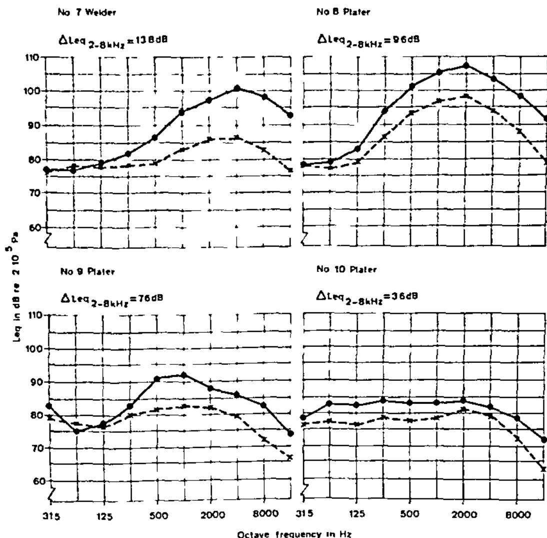


Fig 4 Curves showing the dose as a function of octave bands for the different workers

Fig 5 Curves showing the dose as a function of octave bands for the different workers

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THE RELIABILITY OF BÉKÉSY SWEEP AUDIOMETRY RECORDING AND EFFECTS OF THE EARPHONE POSITION

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Key words Békésy audiometry, Hearing thresholds, Learning effect, Noise-induced hearing impairment, Earphone position, Ear speculum, Pinna.

Abstract The hearing thresholds of subjects with hearing impairment of different severity were tested several times The reliability of the hearing threshold measurements were analysed and compared to the inter-aural variations, the learning effects, the degree of noise-induced hearing impairment and the application of the earphones The main factor determining the reliability of the hearing threshold is the application of the conventional earphone A preliminary test-retest with the earphone adapted to an ear speculum for sound transmission to the external ear canal suggests that the precision of the Bekesy sweep hearing threshold measurement can obviously be increased in the frequency range of 2 to 8 kHz

INTRODUCTION

Repeated hearing measurements with a high precision made at intervals throughout the period of employment could be important in a hearing preservation program. The most suitable method for measuring the hearing threshold levels is probably the Békésy sweep method since it does not require such a skilled operator as conventional pure tone audiometry. The Békésy sweep method also provides a standard threshold procedure with a graphic frequency sweep record. In a retest experiment, Erlandsson et al (1979a) have shown that the standard deviations of hearing thresholds found in a conventional pure tone investigation with one and the same operator are about twice as large as those obtained with the Békésy sweep method. The accuracy and reliability of the hearing threshold levels measured by a Békesy sweep audiometer depends on several factors, e.g. calibration of the audiometer, instruction to the subject before the hearing test, the test conditions and the effect of learning and temporary threshold shifts caused by noise. The first factor can be controlled technically and the effects of the other factors on the hearing threshold have been described by several authors (Burns & Hinchcliffe, 1957, McCommons & Hodge, 1969; Robinson & Whittle, 1973). Other factors which can influence the accuracy and the reliability are shifts caused by the middle ear pressure (Erlandsson et al, 1980), inter-individual variations and the sound pressure transmission from earphone to external ear canal and tympanic membrane.

The aim of this investigation was to increase the precision of the Bekésy sweep hearing threshold measurements in workers exposed to noise. With this in mind we tried to answer the following question: Is the reliability of the test-retest influenced by the following factors. the learning effects, the inter-aural variations, the degree of the noise-induced hearing impairment and the position of the earphone relative to the entrance to the ear canal?

METHOD

A Békésy audiometer, type Demlar 120 was used together with matched TDH-49P earphones with MX-41/AR cushions The hearing tests were carried out in a sound-proofed booth, type T-box Tegnér The duration of a continuous sweep from 0.25 to 10 kHz was 400 s and the rate of intensity change, both increase and decrease, was 2.5 dB $\times$ s^{-1} The tones were presented with a repetition rate of 2.5 pulses/s and with a 50 % duty cycle For a more detailed description, see Erlandsson et al (1979a). The calibration was performed before and

after the investigation according to ISO R 389 (1964). The changes in the output level were less than 1 dB and the frequency drift less than 2 %.

MATERIAL AND PROCEDURE

Seven shipyard workers and two students, aged 31 to 53 years, with hearing impairment of different severity were examined This material was also used in the previous retest investigations (Erlandsson et al, 1979a). To reduce the effect of a possible temporary threshold shift (TTS) all the hearing tests were performed after at least 16 hours after exposure to noise and about the same time in the morning The subjects were also told not to expose themselves to excessive noise during the preceding day.

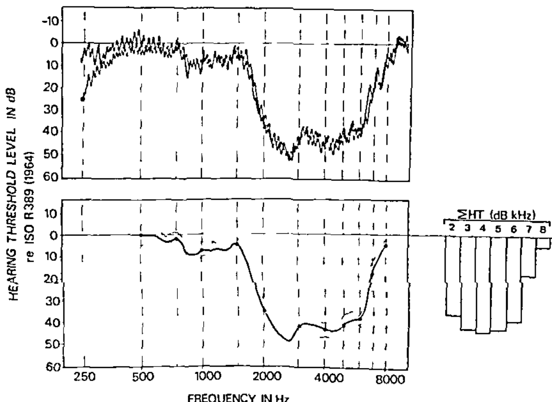


Fig 1 A Békésy sweep recording and calculation of the "midpoint" hearing threshold To the right is illustrated a calculation of the summed hearing thresholds in dB kHz for each integer frequency in kHz from 2 to 8 kHz.

The subject to be tested was placed in the sound-proofed booth and given the following instructions: "You will now hear a beeping sound. As soon as you hear this sound, press the button of the switch and release it as soon as the sound fades away. Never let the sound grow very strong and never let it stay away too long. Press the button as soon as you hear it and release it as soon as it fades away."

The earphones were then placed on the head of the subject at the level of the entrance to the ear canal and in such a way that the headset would stay securely in place. This was carried out by the same operator.

The test signal was then set at 25 dBHL at 0.25 kHz and the subject to be tested was instructed to begin to use the switch a few times. In this way it was possible for the operator to ensure that the instructions were correctly interpreted. The frequency sweep started at 0.25 kHz, stopped at 10 kHz and then returned from 10 to 0.25 kHz. The hearing threshold was then calculated as the mean value between the upper peaks of the double tracing, which represent *the exact moment when the subject could no longer hear the sound*, and the lower peaks the moment when the subject could hear the sound, see fig. 1. The summed hearing thresholds HT_{2-8} in dB for each integer frequency in kHz from 2 to 8 kHz were calculated.

RESULTS

1. The influence of learning effects, inter-aural variations and degree of hearing impairment on reliability of the test-retest

Békésy sweep audiograms of 9 subjects (18 ears) were recorded five times at 1-day intervals. For each subject, the left ear was tested first and after a 10 min pause, the right. The summed hearing thresholds for each integer frequency from 2 to 8 kHz HT_{2-8} were calculated. The pooled estimate of the variance of HT_{2-8} was computed by first obtaining the variance for each ear, after which the mean value of the variance of all the 18 ears was calculated. This was done separately for the left ear, the right ear and both ears, see table I The standard deviation (SD) of HT_{2-8} for the left ear was 17.0 and for the right ear 17 3, and for both ears 17.1 dB.kHz. Consequently, there was no great difference between the ears, as judged from the mean values, although great individual differences were seen.

The HT_{2-8} from each ear were added for each retest day. These values showed no trend towards an increase or a decrease of the hearing thresholds recorded with Békésy sweep audiometry between the different occasions (table I). The number of occasions when the hearing thresholds increased or decreased

TABLE I *The test-retest reliability of the [illegible] during fi[illegible]e days*

Subject	Ear	Age	HT_{2-8} in dB kHz Investigation number 1	2	3	4	5	$\bar{x}$	v	SD
I	Left	31	48	95	72	43	43	60 2	522 7	22 9
II		32	194	196	179	163	166	179 6	234 3	15 3
III	"	35	170	155	179	178	175	171 4	96 3	9 8
IV	"	36	66	85	73	68	113	81 0	374 5	19 4
V		37	366	356	371	368	357	363 6	45 3	6 7
VI		44	139	129	134	124	131	131 4	31 3	5 6
VII	"	45	271	252	269	286	229	261 4	473 3	21 8
VIII	"	46	232	279	289	287	291	275 6	614 8	24 8
IX	"	53	477	477	479	488	510	486 2	197 7	14 1
Σ			1963	2024	2045	2005	2015	2010 4	2590 2	16 96
I	Right	31	48	57	77	71	43	59 2	212 2	14 6
II		32	166	171	178	157	157	165 8	82 7	9.1
III		35	98	99	110	106	87	100	77 5	8 8
IV	"	36	79	73	42	65	78	67 4	232 3	15 2
V	"	37	195	237	229	183	224	213 6	543 8	23 3
VI		44	217	171	187	224	226	205 0	606 5	24 6
VII	"	45	348	335	341	321	335	336 0	99 0	10 0
VIII	"	46	372	386	359	387	415	383 8	435 7	20 9
IX	"	53	423	450	457	470	473	454 6	400 3	20 0
Σ			1946	1979	1980	1984	2038	1985 4	2690 0	17 29
Σ	R+L		3909	4003	4025	3989	4053	3995 8	5280 2	17 13

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Application of TDH 49P with MX 41/AR cushions			
On Off	On Off (1)	Fixed (2)	Variable (3)

Subject I			On Off	On Off (1)	Fixed (2)	Variable (3)
	Right	o----o	SD 14 6	4 6	4 9	11 4
	Left	x——x	SD 22 9	24 5 (71 1)	3 6	29 2
	Left	▲——▲	SD	59 1		

HT 2 8 (dB kHz)

200, 150, 100, 50, 0

(X)

1 2 3 4 5 — 0 1 2 — 0 1 2 — 0 1 2

Subject IV			On Off	On Off (1)	Fixed (2)	Variable (3)
	Right	o----o	SD 15 2	8 9	7 8	10 3
	Left	x——x	SD 19 4	23 4	6 3	8 9
	Left	▲——▲	SD	24 2		

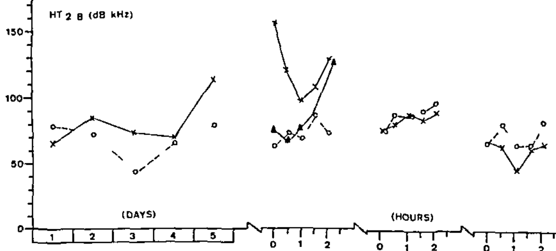


Fig 4 The test-retest of the $HT_{2-8\ kHz}$ during four sequences with on off procedure, fixed and different positions of the earphones *r* * *Bekesy sweep recording of t*

whole test-procedure, e.g a fixed position Third The earphones were placed in five different positions: at the level of the entrance to the ear canal, 7 mm above, below and to either side of the first position.

The summed hearing thresholds between 2 and 8 kHz, HT_{2-8}, are shown in fig 4 together with the five days' retest results for these persons. The standard deviation of the summed hearing thresholds, HT_{2-8}, and of the hearing thresholds at the individual frequencies are shown in table II The mean value of the standard deviations of HT_{2-8} for the five test days' experiment was 18.3 dB kHz and for the two hours' experiment was 17.7 dB.kHz (first sequence). The difference was negligible. The same result was obtained for the different earphone positions, 17.1 dB.kHz (third sequence). It is also clear that for the fixed position (second sequence) the standard deviations were smaller for test frequencies higher than 1 kHz.

Comments In the test-retest during the first sequence the fourth measurement on the left ear of subject No 1 was excluded from the calculation of the standard deviation. The ear canal might have been closed by the earphone during this measurement, which could explain the high HT_{2-8}-value. Some ears are undoubtedly more sensitive to the position of the earphones. The great standard deviation with on-off procedure of the earphones for the left ears of the subjects was reproduced in a retest study, see fig. 4.

TDH49P earphones with ear specula In order to verify further the importance of the application of the earphones an experiment was performed where the earphone was coupled to a standard ear speculum with a diameter of 5 mm A special headset was used and the speculum was inserted in the subject's ear canal before each of the five Békésy sweep recordings (fourth sequence) The reliability of the hearing thresholds in the two subjects is shown in table II, which is in accordance with the small standard deviations when the earphone was fixed in position (second sequence). The mean hearing thresholds in the fourth sequence were improved for frequency over 2 kHz relative to corresponding mean hearing thresholds in sequences 1-3, see fig. 5.

D I S C U S S I O N

According to Burns & Hinchcliffe (1957) and Robinson & Whittle (1973) there is a hearing threshold improvement of 1 to 2 dB at most frequencies by repeated Békésy audiometry after an interval of one week and 15 months, respectively. This improvement has been ascribed to a learning effect. However, our results showed no tendency towards improvement of the summed hearing thresholds from 2 to 8 kHz between different occasions. This learning

Table II *The standard deviation of hearing threshold levels for different earphone applications. First sequence, one Békésy sweep recording (Subject No I, left ear), has been omitted because of an exceptional deviation. In test-retest with intervals of 15 min the same subjects I and IV were used*

Earphone	Kind of application	Time x Δt	Subjects/ ears	SD of hearing threshold level in dB at frequency in kHz									
				0.5	1	2	3	4	5	6	7	8	HT_{2-8}
TDH49 + MX-41/AR	On-off	5 x 24 h	9/18	3.1	2.4	3.5	2.7	3.1	3.4	3.4	3.4	4.2	17.1
TDH49 + MX-41/AR	On-off	5 x 24 h	2/4	2 8	2.0	4.3	3.1	2.8	4.3	3.2	3.2	3.9	18.3
TDH49 + MX-41/AR	On-off(1)	5 x 15 min	2/4	2.4*	1.5*	3.1*	3.1*	2.8*	2.3*	3.8*	4.1*	2.8*	17.7*
TDH49 + MX-41/AR	Fixed (2)	5 x 15 min	2/4	2.3	1.3	2.2	1.8	1.3	1 0	1.6	1.9	1.6	5.8
TDH49 + MX-41/AR	Variable(3)	5 x 15 min	2/4	3.4	1.4	2.7	3.4	4.4	6.0	4.5	4.5	4.0	17.1
TDH49 + Ear speculum	On-off(4)	5 x 15 min	2/4	1.7	1.4	1.3	1.2	1.0	1.8	1.8	2.6	1.4	5.3

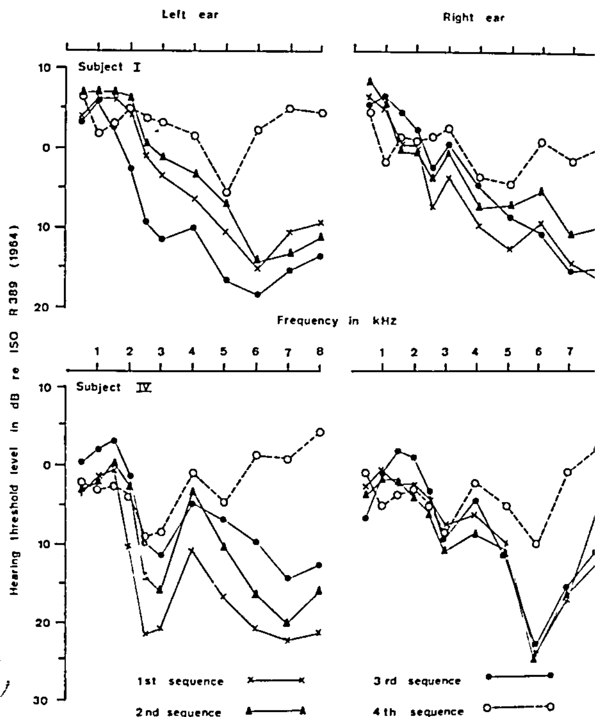


Fig. 5. The mean hearing thresholds at different frequencies at the test-retest with Békésy sweep audiometry of two subjects. TDH-39P earphones with MX-41/AR cushions are used in the sequences 1-3 and TDH-39P earphones with ear specula in sequence 4

effect was demonstrable only during the first 0.5 min of each Békésy sweep recording, i e. a hearing threshold difference between the up and down frequency sweep in the range from 0.25 to 0.5 kHz, see fig 1.

The temporary threshold shift caused by small middle ear pressure variation (< 5 cm H_2O) relative to ambient pressure can be ignored by using the summed hearing thresholds between 2 and 8 kHz, HT_{2-8}, according to Erlandsson et al (1979b) and Erlandsson et al (1980). The middle ear pressure in the test-retest during the five days was not checked, but the influence of this pressure on the reliability of HT_{2-8} is rather small. In test-retest sequences during two hours the absorbed middle ear gas contributes only slightly to the middle ear pressure variation of about 1 cm H_2O and this contribution can thus be ignored.

In order to reduce the effect of a temporary threshold shift caused by industrial noise, all subjects in the present investigation had avoided occupational noise for 16 hours before a hearing test. Ward (1975) reported that subjects with 24 hours of exposure to noise levels of 75 and 80 dB centered at 4 kHz had completely recovered their hearing thresholds 16 hours after the exposure In this experiment where the noise level is about 85 dB, the subjects required 24 hours for complete recovery Therefore, our subjects were told to use hearing protectors the whole time and to avoid exposure to excessive noise during the preceding day.

The reliability of HT_{2-8} between the left ear and the right in each subject showed a great difference and no correlation with the degree of hearing impairment. The influence of the earphone position on the reliability of the Bekésy sweep hearing threshold measurements must therefore be a determining factor

Bekésy (1947) reported that in the frequency region above 5 kHz the resonance of the ear canal and the earphone affect the reproducibility of the hearing threshold and that the resonances change each time the earphone is applied anew

Present test-retest studies showed that the position of the earphone relative to the entrance to the ear canal has pronounced effects on the hearing threshold measurements for test frequencies higher than 1 kHz. The standard deviation of HT_{2-8} for five marked positions (third sequence) gives the same result as for normal earphone application (first sequence). During fixed position (second sequence), on the other hand, the test-retest gives a smaller standard deviation both of summed hearing thresholds between 2 and 8 kHz and the hearing threshold for each frequency higher than 1 kHz, see fig 4 and table II This is valid for all test-retests except for the right

ear of subject No 1 in the first sequence. The reliability of HT_{2-8} with normal earphone application was about 17 dB.kHz, compared with about 6 dB.kHz when the earphones were firmly placed over the subjects' ears during the whole test-retest. This difference shows that the position of the earphone is an important factor in determining the precision of the hearing threshold with Békésy sweep audiometry. The standard deviation when the earphones are in a fixed position is so small that uncertainties in the calibration of the audiometer and in the calculation from the graphic frequency sweep probably are the main factors.

Although the outer ears of the subjects in the present study were regarded as otologically normal the headset presses the cushions of the earphones against the pinnas with a mechanical pressure, which can vary from one occasion to another. A cushion contact with the surface of the pinna gives a variation in the shape of the pinna. This may affect the transformation of the sound pressure to the ear canal entrance. Variations in pinna size are most effective on the sound pressure level in the outer ear above approximately 6 kHz, where higher-order acoustics modes are strongly excited in the pinna (Kuhn, 1979). The cushion can also come in contact with the tragus, which affects the entrance opening to the ear canal. This may explain why the standard deviation of the hearing thresholds for nearly all the frequencies and of the HT_{2-8}-value was higher for the two sequences where the position of the earphones was varied, than for the sequence with the fixed earphones. The mechanical pressure against pinna may be an important factor for the sound transmission.

A preliminary test-retest with the ear speculum device denotes that the precision of the Békésy sweep hearing threshold measurement can be quite obviously increased in the frequency range of 2 to 8 kHz. The reliability of the hearing thresholds during on-off procedures with the earphones adapted to ear specula was the same as when the cushions of the earphones were firmly placed over the outer ears during the whole test-retest, see table 11. This confirms our assumption that the placing of the earphones is of great importanc However, the new earphone device gives a threshold gain for the frequencies higher than 2 kHz (fourth sequence), see fig. 5. Such a threshold gain may be explained by a change in the acoustical condition of the ear canal, when an ear speculum is inserted. The normal meatus is a resonator about 2.7 cm long with the fundamental resonance frequency of approximately 4 kHz and an average gain at this frequency of about 10 dB (Weiner & Ross, 1946).

CONCLUSIONS

Observations made in the present study showed that the reliability of the hearing threshold measurements made by Békésy sweep audiometry partially depends on the application of the earphones. This may be explained by the fact that the mechanical pressure of the earphone cushion deforms the pinna and affects the transmission of the sound pressure to the ear canal entrance. A preliminary test-retest with an ear speculum adapted to the earphone for the transmission of the sound from the earphone to the external ear canal suggests that the precision of the hearing threshold recorded by a Békésy sweep audiometer can obviously be increased. This facilitates earlier detection of hearing impairment caused by noise and makes it possible to control the efficiency of different hearing protectors.

ACKNOWLEDGEMENTS

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DIFFERENT KINDS OF NOISE

AIR CELL DAMAGE IN THE [illegible]

DUE TO

DIFFERENT KINDS OF [illegible]

by

B.Erlandsson, H.Håkanson, [illegible]
P.Nilsson and J.[illegible]

ort dot at 79%
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Pluznik"
ad "Federspil"
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HAIR CELL DAMAGE IN THE GUINEA PIG DUE TO DIFFERENT KINDS OF NOISE

Errata

Acta Otolaryngol Suppl. 367.

Page 12, line 4, "OsO_2" *should read* "OsO_4"

Page 24, graph 120 dB SPL, segment 5, *Insert dot at 79%.*

Page 25, graph 102 dB L_{eq}, segment 4, *Delete dot at 55%.*

Page 28, graph Series II, 120 dB SPL, *Insert dot at 27%*

Page 32, line 10, "Pluzmik" *should read* "Pluznik"

Page 32, line 36, "Federspiel" *should read* "Federspil"

Page 40, line 18, "Hamernick" *should read* "Hamernik"

Page 43, Between line 15 and 16. *Insert reference:* Ůlehlová, L. & Voldřich, L. 1978. Idiopathic hair cell loss in the guinea pig. *Arch Otorhinolaryngol* 221, 7.

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1. INTRODUCTION

Occupational hearing loss is a well-known result of exposure to noise and has for many years attracted wide attention. Several attempts have been made to find a way of measuring such exposure and expressing its noxiousness by a single number.

One such simplification (Ward, 1979) is the speculation of the "equal-energy principle", which postulates that permanent damage to the hearing is related to the total sound energy (a product of sound intensity and duration). The equal-energy principle thus postulates that equal noise energy causes equal hearing losses. The equal-energy concept allows a 3-dB increase in sound pressure level (SPL) for each halving of the duration below 8 hours (U.S. EPA, 1973). The study of Burns & Robinson (1970) proposed the extension of the equal-energy principle to overall durations of daily occupational exposures and Atherley & Martin (1971) extended the concept also to impulse noise by the application of the "equivalent continuous sound level" (L_{eq}) having the same energy as the actual time-varying noise. The equal-energy concept has been adopted partly because of its simplicity and partly because of experimental evidence (Martin, 1973). Other observations of occupational noise (Passchier-Vermeer, 1971; Bruel, 1976) indicate a higher risk of hearing loss by impulse sounds than the equal-energy concept would imply. Scheiblechner's analysis (1974) of a selected material of workers exposed to noise also refused the equal-energy principle in almost all cases.

Human population studies of the relation between noise exposure and hearing loss are, however, difficult to perform because of the complexity of the measurements Studies are influenced by differences within the population, by difficulties in performing representative individual noise measurements by differences in the noise protection efficiency and non-occupational noise exposure and by variations in the audiometric measurements.

It was therefore thought reasonable to study the problem experimentally in animals, where the noise parameters, the pathological physiology and anatomy can be controlled Experimental studies of the relationship between noise and damage to the hearing and the inner ear have been performed for many years. Guild s (1921) description of a method for graphically reconstructing the cochlea paved the way for more extensive studies using histological as well as electrophysiological methods Davis et al, 1935, Lurie et al, 1944; Alexander et al, 1951, Covell, 1953, Davis et al, 1953, Schuknecht, 1953)

Many investigations on animals exposed to noise are not comparable to studies of the effect of occupational industrial noise. Some experiments have involved

short exposures at high sound levels (Beagley, 1965) and in other instances white noise (WN) or different kinds of band-passfiltered noise have been used (Spoendlin, 1971), which might introduce non-linear distortion products (Bilger & Hirsh, 1956), making such noise incomparable to industrial noise (Ward & Nelson, 1971). In many publications no information is given of the acoustic situation other than the sound level, and when impulse noise has been used, the records concerning the time and frequency pattern of the acoustics of the impulse noise are mostly insufficient Moreover, many experiments have been performed on only one or a few animals ignoring the inter-individual variation Experiments have also been performed on different species which complicates comparisons of the conclusions

This means that systematic studies of the relation between noise at industrial levels and damage to the ear and hearing in animals, which might be of value for interpreting the equal-energy concept, are rather few Important contributions have been made by Ward & Nelson (1971) and Blakeslee et al (1978) Hamernik et al (1974) and Hunt et al (1975) made observations indicating that the equal-energy concept is not valid when the impulse noise is superimposed upon a continuous sound

The aim of this work was to study the relationship between noise exposure and histological inner ear damage under conditions filling the following requirements

a The sound levels used should be comparable to those in a workshop
b The sound levels and/or duration of exposure should be systematically varied
c The acoustic situation should be thoroughly analyzed and continuously controlled
d The amount of animals used should be large enough to minimize inter-individual variations

2 MATERIALS AND METHODS

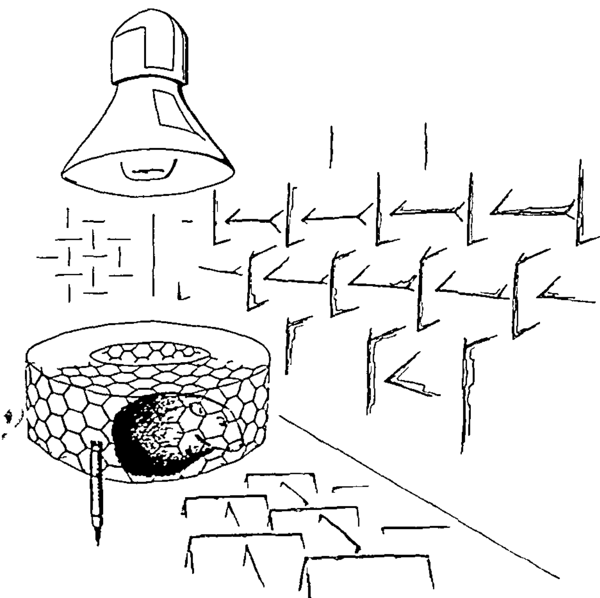
2 1 EXPERIMENTAL FACILITIES

2 1 1 *Localities*

All exposures to sound were performed in an anechoic chamber measuring 4 25 x 2 25 x 2 15 m, constructed with 0.5 m high, sound absorbing fiber glass wedges

The sound was directed from above on to a circular wire mesh cage with an inner diameter of 15 cm and an outer diameter of 40 cm, in which the animals were able to move about freely, fig 1 The cage was adjusted in the sound

field so that the Peak Sound Pressure (PSP) and L_{eq}-values were within ± 0 25 dB in each of the four quadrants of the cage No significant change in PSP or L_{eq} could be observed when the cage was removed from the sound field with the microphone positioned at the level of the animals' ears The influence on the sound level caused by the animals did not exceed 1 0 dB



Arrangement of the animal cage and the exponential horn in the anechoic chamber

2 1 2

In all the experiments the sound was measured with a Bruel & Kjaer (B & K) Impulse Precision Sound Level Meter (SLM) 2209 equipped with a B & K 4165 1/2" microphone The signal from the SLM was also fed to a Nicolet 1090 A Digital Memory Oscilloscope and recorded in a 4096 word memory Parallel to the oscilloscope the signal was fed to a B & K 4423 stationary Noise Dose Meter, where the L_{eq} was monitored with a 1 ms integration time The whole system was carefully calibrated with a B & K 4220 pistonphone

2 1 3

2 1 3 1 Pure-tones were used as continuous sound stimuli The sinusoid output from an oscillator was amplified by a RCF AM 4120 power amplifier and then fed to a RCF exponential horn D 5075 The horn was placed above the circular cage, see fig 1 Two sinus frequencies of 3846 Hz (3 85 kHz) and 1333 Hz (1 33 kHz) were used

The frequencies and shape of the two pure-tones were continuously controlled with the Nicolet oscilloscope At regular intervals the signal was memorized in a Memoscope Digital Camera Tape recorder The frequencies did not fluctuate beyond two decimals and no modulation of the signal could be seen on the oscilloscope The distortion of the 3 85 kHz tone was checked separately with a Hewlett Packard (HP) 4333A Distortion Analyzer The first harmonic was found to be 0 6 % of the fundamental frequency (f_0) This distortion could not be detected on the oscilloscope No other frequency components could be found The use of A weighting filter increased the meter reading by 0 9 dB at 3 85 kHz and by 0 6 dB at 1 33 kHz at the sound intensities used in the experiments

2 1 3 2 *Impulse sound stimulus* To fill the demands of an impulse sound allowing variation of the height of the pressure peak, the duration of the total pulse, the main frequency content and the repetition rates of the impulses, a motor hammer was constructed (fig 2) The construction of the hammer has been described by Erlandsson et al (1980) The hammer-head is filled with lead to prevent it from rebounding To achieve constant sound pressure patterns it is important that the hammer head must hit flat on the plate

The continuous noise from the motor and from other parts of the hammer is below 70 dB SPL and thus unimportant compared to that of the hammer blows The speed of the motor can be varied between 10 and 120 rpm without influencing the action of the hammer It is thus possible to change the L_{eq} by changing the rate of impulse production without changing the PSP The wooden plate of the generator was placed 50 cm above the animal s cage



Fig 2 The impulse sound generator in the anechoic chamber

The reliability of the impulse generator was controlled with the B & K 4423, and at regular intervals the noise dose was read It was possible to operate the hammer for more than 72 hours continuously with changes of at most 0 2 dB in the L_{eq}-value The reproducibility of the impulses was checked with the digital memory oscilloscope

Figure 3 shows two impulse configurations, the first at the start and the second, 50 000 hammer blows, later There was no difference in the pressure pattern during the first 7 ms, where about 85 % of the energy content was located Only in the later oscillations were there any differences The PSP of the impulse was 131 5 dB SPL during all exposures The B duration (the total time that the envelop of the sound pressure fluctuations is within 20 dB of the maximum PSP) was 54 ms The frequency content of the impulse sound was checked by Fast Fourier Transform (FFT) analysis with the use of a Nicolet 444 A Mini Ubiquitus FFT Computing Spectrum Analyzer Frequency peaks were found at 780, 990, 1120, 1190 and 1430 Hz (fig 4)

A weighting of the impulse sound reduced the L_{eq} by 0 4 dB In the FFT analysis the 50 Hz peak was reduced 10 dB and the 150 Hz peak 2 7 dB by A weighting

Fig. 3. Pressure pattern during the first milliseconds of the impulse of the hammer blow. Comparison is made with the pressure pattern of an impulse recorded 50 000 hammer blows later.

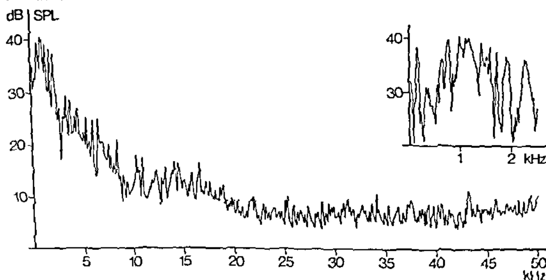


Fig. 4. FFT analysis of 256 averaged impulses. Inserted in the figure is an enlargement of the first 2.5 kHz.

There was no significant difference between 700 and 5 000 Hz and above this region the A-weighting successively reduced the intensity of the spectrum. At 10 000 Hz the reduction was 2.5 dB.

2.2. EXPERIMENTAL SERIES

Four different series of experiments were performed and the number of the animals and other parameters are given in table I.

Series I

Five groups of animals, each consisting of 5 guinea pigs, were exposed to a 1.33 kHz continuous pure-tone for 6 hours at one defined sound intensity. The difference in exposure between the groups was changes in the sound intensity.

Series II

The same as series I, but with a pure-tone frequency of 3.85 kHz.

Series III

In each of 3 different experiments 5 guinea pigs were exposed to the sound from the impulse generator for 6 hours. The experiments differed in that the L_{eq} was changed by varying the rate of impulses per min but without change of the character of the impulse.

Series IV

In this series the impulse generator was used. The sound pressure pattern and the number of hammer blows per min were kept constant, but the exposure time was varied

2.3 ANIMALS

Pigmented half-inbred female guinea pigs (father pigmented inbred stock since 1928, mother Duncin-Hartley inbred since 1971) with melanotic (dark) eyes, aged 6-7 weeks (270-315 g) at the time of exposure to noise were used. Preyer's reflex was controlled before and after the exposure. After the experiment the animals were caged for another 4 weeks and then killed

All together 85 animals, including 5 controls, were used. The control animals were not exposed to sound. They were of the same strain, breed and age and were treated in the same way as the experimental animals. In all the experiments the guinea pigs were confined in the circular cage and with free access to their ordinary food and water. No feeding problems could be detected even during the 48-hour experiment. The animals could move about freely and were found to change their positions in such a way that the exposure was assumed to be envenly distributed

Table I *experim t series showing composition of parameters and number of animals*

Series No	Sound	No of animals in each exposure	No of exposures	Constant parameters in each exposure	Variable
I	PT	5	5	Time 6 h f 1 33 kHz	Intensity 102,108,114,117,120 dB SPL
II	PT	5	5	Time 6 h f 3 85 kHz	Intensity 102,108,114,117,120 dB SPL
III	Impulse	5	3	Time 6 h PSP 131 5 dB SPL f FFT(fig 3)	Intensity 92 7, 97 7, 102 0* dB L_{eq}
IV	Impulse	5	3	PSP 131 5 dB SPL f FFT(fig 3) I 102 0 dB L_{eq}	Exposure duration 6 h*,12 h,24 h,48 h
	Total animals	80			

Abbreviations
PT = Continuous Pure-Tone
FFT = Fast Fourier Transform
PSP = Peak Sound Pressure
SPL = Sound Pressure Level
f = Frequency
I = Intensity
L_{eq} = Equivalent continuous sound level

* same exposure

2.4 MORPHOLOGICAL ANALYSIS

2.4.1 *Preparation*

Both ears of each animal were prepared for further analysis. The cochleae were fixed in 1 % OsO_2 in phosphate buffer, pH 7.4 (Rhodin, 1954) by perfusion. After having been rinsed and dehydrated, one of the ears was stored for electronmicroscopic (scanning) studies and the other was embedded in epoxy resin (Wersall, 1956, Luft, 1961). After polymerisation the cochlea was cut in two halves with a 0.1 mm thick circular saw under a preparation microscope. The saw was centered in a plane through the modiolus and through the round window. The segments from each half of the cochlea were mounted in epon for surface studies (Neubert, 1952, Engstrom et al, 1966, Ernstsson, 1972, Spoendlin & Brun, 1974). From each cochlea, nine such semicircular slices of increasing length from the apex (segment 1) to the base (segment 9) were mounted. Existing and missing hair cells in the whole cochlea were counted with the aid of a waterlens and an interference contrast microscope (Spoendlin & Brun, 1974).

2.4.2 *Cell counting*

The hair cells in 70 of the 85 guinea pig cochleae were counted by one technician (B.B.), those in the remaining 15, by another (W.R.) with the assistance of B.B. in the examination of the damaged areas and segments. The cells in each of the four rows were counted and each cell was evaluated as present or missing, and the results were calculated for each segment. All nine segments were successively examined in this way.

2.4.3 [illegible]

Cells, with identifiable cellbody and cuticula, were registered as remaining cells. Distinctive scarformation formed by the convergence of adjacent phalangeal cells was regarded as sign of missing hair cell in accordance with Engstrom et al (1966), Poche et al (1969), Stockwell et al (1969) and Coleman (1976a,b).

2.4.4 [illegible]

In the animals of the series I-IV the most common sign of damage was the loss of outer hair cells (OHC-loss). This damage was more scattered within the cochleae exposed to lower sound intensities. A typical feature of the damage at higher intensities was collapse in the regular cell pattern due to loss of outer pillar cells. Another sign of damage was rifts, extending into the row of the inner hair cells (IHC), sometimes accompanied by loss of pillar cells. More extensive areas devoid of hair cells were seen, and more extensive IHC-

degeneration was accompanied by loss of cochlear neurons. The number of missing hair cells was estimated even in the areas of total destruction. Typical appearances of the damage are illustrated in figs. 5-14.

2 4 5. [illegible] of [illegible]

After adjustment of the slices of the cochlea according to Kimura (1965) and Ylikoski et al (1974) the length of each segment was checked with Coleman's (1975) method for cochlear length estimation using the number of both IHC and OHC. Owing to angular differences in the plane of the saw from the modiolar plane the segments could vary in dimension. To find out how the hair cell loss distribution varied within the cochlea, the lengths of segments of the cochleae from three exposures (15 animals) were computed. A mean length of each segment was calculated and used for the illustrations (figs.15-18).The relation between segment number and turn number, which is used by other authors, is given in table IIa.

The most apical segment (segment 1) has a varying organization of the OHC-distribution This segment is also very difficult to prepare in such a way that remaining OHC can be counted In this study the OHC of the first segment could be counted only in 17 of the 85 cochleae. The counts of segment 1 were therefore not used.

The length of the round window segment (the 'hook"), the most basal segment, varied more than the other segments This was caused only to a minor degree by an angular rotation of the saw plane in relation to a plane incorporating the round window and the modiolus The major reason was the difficulty to prepare the hook without damaging it

2 5 EVALUATION

The most prominent sign of damage was the OHC-loss. The appearance of the OHC-loss made it possible to treat the results numerically. This was not possible with the other signs of cochlear damage, which were therefore not taken into further account in the current work.

In all the series the results were calculated in two ways. First the OHC-loss percentage was calculated for all three rows of each segment and the results are given for each animal and as mean values for the five animals in each exposure group The results are plotted in figs. 15-18. Then the total OHC-loss percentage from segments 2-8 was calculated for each cochlea and is given for each animal and as a mean value of the five animals in each exposure group. The results are plotted in figs. 19-21.

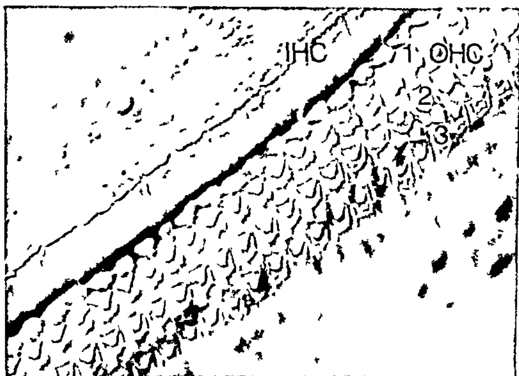


Fig 5 Interference-contrast micrograph, 8 2 mm from base of the cochlea in guinea pig M 91, exposed to impulse noise for 6 hours at 102 dB L_{eq} In this part of the cochlea the hair cell population is normal x 700

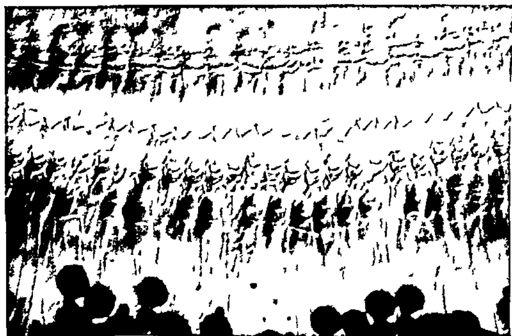


Fig 6 Interference-contrast micrograph, 15 0 mm from base of the cochlea in guinea pig M 101, exposed to impulse noise for 6 hours at 97 7 dB L_{eq} Six OHC are missing in the second and third rows The total OHC loss in the segment was 7% All IHC are present x 650

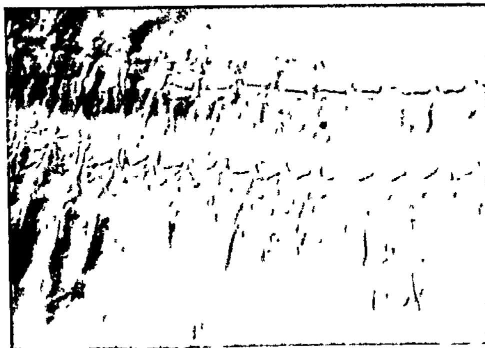


Fig 7. Interference-contrast micrograph, 15.2 mm from base of the cochlea in guinea pig M 85, exposed to impulse noise for 12 hours at 102 dB L_{eq}. Half of the cells in the second and almost all cells in the third OHC-row are missing. The total OHC-loss in the segment was 22 %. All IHC are present. x 1 100

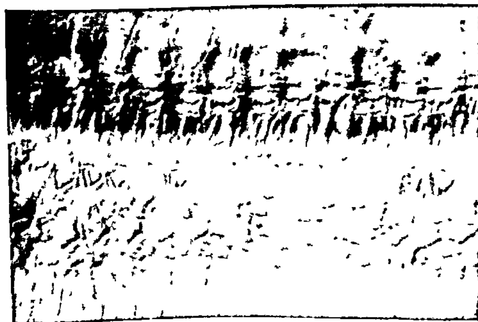


Fig. 8 Interference-contrast micrograph, 13.6 mm from base of the cochlea in guinea pig M 72, exposed to impulse noise for 48 hours at 102 dB Leq [illegible] OHC loss with onl[illegible] remaining cells in the first [illegible]

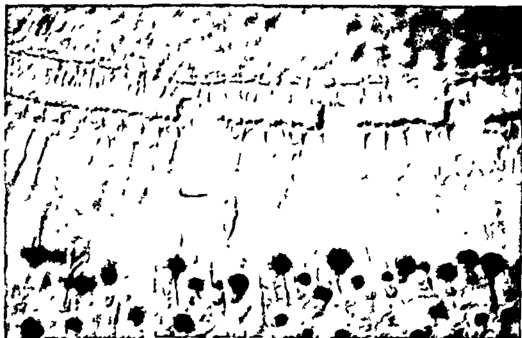


Fig.9. Interference-contrast micrograph, 13 3 mm from base of the cochlea in guinea pig M 71, exposed to impulse noise for 48 hours at 102 dB L_{eq} The OHC-loss is very extensive with only a few remaining cells and there are three collapses due to missing outer pillar cells. On the right hand the typical scar formation formed by converging Deiters' cells are observed. The total OHC-loss in the segment was 75% All IHC remain. x 650

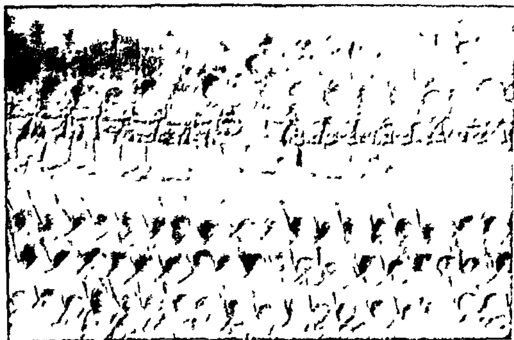


Fig 10 Interference-contrast micrograph, 4 0 mm from base of the cochlea in guinea pig M 135, exposed to pure-tone 3 85 kHz for 6 hours at 102 dB SPL A rift is penetrating the IHC-row The total OHC-loss in the segment was only 1% x 650

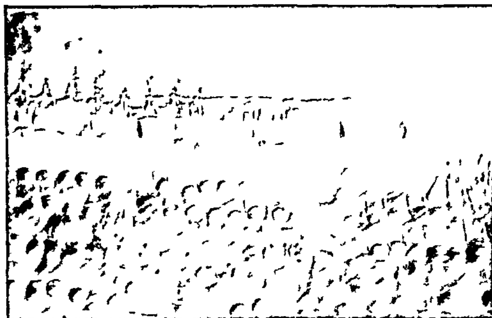


Fig 11 Interference contrast micrograph 8 9 mm from base of the cochlea in guinea pig M 136, exposed to pure tone 3 85 kHz for 6 hours at 102 dB SPL The figure shows moderate OHC-damage and three rifts due to outer pillar cell losses The total OHC loss in the segment was 44% All IHC remain x 650

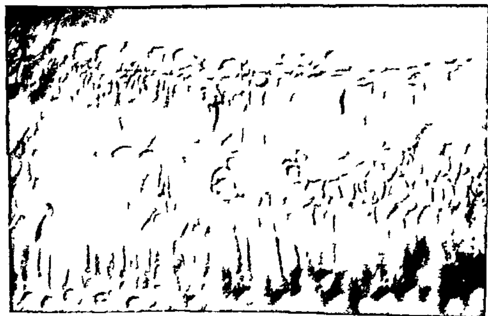


Fig 12 Interference-contrast micrograph, 11 3 mm from base of the cochlea in guinea pig M 83 exposed to pure-tone 3 85 kHz for 6 hours at 120 dB SPL Only a few OHC remain and two rifts penetrate into the IHC-row The total OHC-loss in the segment was 60% x 650



Fig. 13. Scanning electron micrograph, 17.8 mm from base of the cochlea in guinea pig M 73, exposed to impulse noise for 48 hours at 102 dB L_{eq} The OHC all remain in the figure but there are slight irregularities in the arrangement of the hair cell rows as well as signs of hairfusion in some of the hair bundles. The total OHC-loss in the segment was 9%. x 3 000 .



Fig 14 Scanning electron micrograph, 15 2 mm from base of the cochlea in guinea pig M 73, exposed to impulse noise for 48 hours at 102 dB L_{eq} Several OHC in the third row are missing and Deiters' cells have closed the defects Two hair cells (arrows) have missing hair bundles but the cell cuticle is still present x 3 000

The relationship between the length of the basilar membrane and the frequency localization was taken from Greenwood (1961), who described a function $f \quad A (10^{ax} - 1)$, which is derived from Bekesy s (1960) elasticity data In this function, where f is the actual frequency and x is the distance in mm from the helicotrema to the region of maximum displacement on the basilar membrane at this frequency, the values of the two constants in these guinea pigs were calculated to A 400 and a = 0 1105 This frequency scale is also given in figs 15-18

2 6 AGING

The influence of aging was estimated from an analysis of the OHC-loss in five non-exposed control animals They were of the same strain, breed and age as the experimental animals The results are given in tables IIa and b

The mean values of the total numbers of present and missing OHC and OHC-losses for each segment are given in the first two lines of table IIa The next line gives the percentages of OHC-loss The standard deviations were also calculated The total OHC-losses in segments 2-8 and the variations are given for each animal in table IIb In this computation the hook was not included as it was damaged in one cochlea in association with the preparation procedure In the other animals the hook showed no OHC-damage but varied widely in size The results given in table IIa (third line) are given also in figs 15-18 and the results given in table IIb (last line) are plotted in figs 19-21

3 R E S U L T S

3 1 CONTROL ANIMALS

The total number of OHC in each segment from segment 8 to segment 2 decreased with the size of the segments towards the apex (table IIa) The numbers given in the second line are small and refer to the spontaneous hair cell loss, which is reflected in the low OHC-loss percentage (third line) There was a slight rise in OHC-loss percentage towards the apical part of the cochlea giving a mean of 1 64 % in segment 3 and 1 03 % in segment 2 The highest value for any animal was 3 7 % in segment 3 and 4 1 % in segment 2

The mean total OHC-loss for segments 2-8 in the five controls was 30 OHC The individual values and the standard deviations are given in table IIb The total OHC-loss percentage averaged 0 58 $\pm$ 0 27 % As no damaged cells could be found in these parts of the hook, which were undamaged afther the preparation, the true loss percentage was probably still smaller

Table II *COVTROL GUINEA PIGS (n 5)*

a) Mean cochlear distribution of Outer Hair Cell (OHC) loss

		Base ⬅ 9	8	7	6	5	4	3	➡ 2	Apex 1
Segment No		9	8	7	6	5	4	3	2	1
Turn No		RW(Hook)	T_1		T_2		T_3		T_4	
Total OHC No	X	860	1351	947	771	667	643	514	395	
	SD	± 188	± 185	± 182	± 143	± 113	± 163	± 80	± 130	
OHC-loss No	$\bar{X}$		1 8	4 2*	4 4	6 0	2 6	8 6	2 75	
	SD		± 2 05	± 3 11	± 4 39	± 4 47	± 1 82	± 7 96	± 5 5	
OHC-loss %	$\bar{X}$		0 13	0 43	0 63	0 92	0 38	1 64	1 03	
	SD		± 0 17	± 0 32	± 0 62	± 0 66	± 0 23	± 1 36	± 2 06	

RW = Round window segment

b) Total hair cell loss

Guinea pig No	140	141	142	143	144	140-144 $\bar{x}$	140-144 SD
Total OHC-count Segment 2-8	4983	5272	5622	5058	5105	5208	± 255
Total OHC-loss Segment 2-8	20	39	23	18	50	30 00	± 13 91
OHC-loss %	0 40	0 74	0 41	0 36	0 98	0 58	± 0 27

3 2. ANIMALS EXPOSED TO NOISE

3 2 1 *General pattern of damage*

OHC-losses were found in all the series exposed Even the values for the hair cell losses in the animals exposed to the lowest intensities/shortest duration significantly exceeded those of the control animals according to a Mann-Wittney U-test ($p<0\ 01$). It is therefore probable that these losses were noise-induced

IHC-losses were recognized in all the series and were accompanied by neural degeneration in the highest intensities of the pure-tone series Rifts were common in the pure-tone series, but were not seen in the impulse series

3 2 2 *Pattern of damage distribution*

3 2 2 1 *Pure-tone series* The results in the series where the animals were exposed to the pure-tone (f_0 1 33 kHz, 6 h, varying intensity) are given in fig 15 The mean value of the OHC-damage in each segment of the cochlea showed a peak at the apex and in segments 5 and 6 The mean value increased in these segments for the 114 and 117 dB SPL intensities With increasing intensity damage in segments 4 and 7 became more pronounced The overall pattern in the figure showed increasing percentages of OHC-loss involving a wider area around the most damaged segment with increasing intensity of the stimulus Another feature of increasing intensity was the much wider range of OHC-loss percentage between the different animals

The site of the damage peak in the cochlea was in fairly good agreement with the frequency localization, which for 1 33 kHz should be 13 5 mm from the base The results in the pure-tone series, where f_0 was 3 85 kHz, the duration was 6 hours, and the intensity was varied, are shown in fig 16 For all exposures in this series the damage was centered in segment 7, which is also in good agreement with the frequency axis In segment 7 the damage was roughly of the same order from 102 dB to 117 dB SPL At 120 dB SPL there was an increase to an almost total damage From 114 dB SPL damage occurred in segment 6 (2 0 - 3 5 kHz) and at 120 dB SPL, the segments 5, 8 and 9, surrounding the center of the damage (segment 7), also showed 20-40 % damage This series also demonstrated a widening range of damage between the different animals when the intensity was increased

3 2 2 2 *Impulse noise series* The results in the impulse noise series III, where the pressure pattern and the duration (6h) were kept constant but where the L_{eq}-value was varied by changing the hammer rate, are shown in fig 17 Compared to the control animals, all the exposed animals show a damage of about 10 % in the two most apical segments 2 and 3 In segments 4-7 there

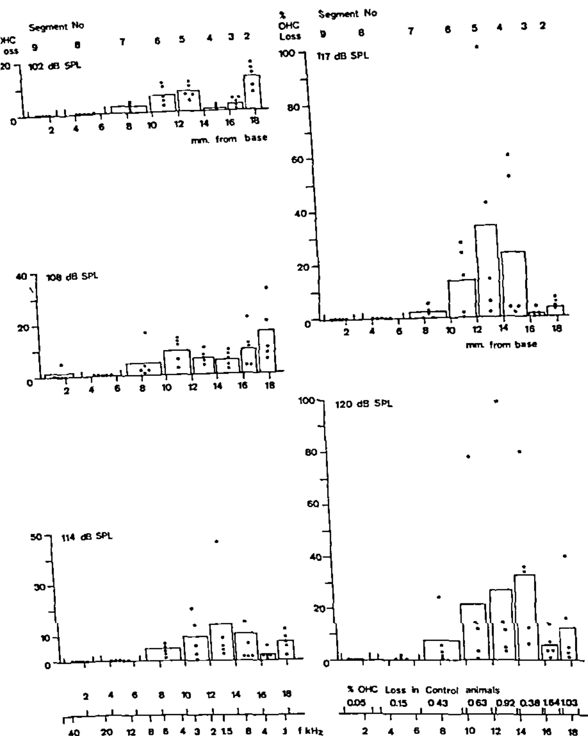


Fig 16 Mean value (columns) and individual values (dots) of OHC-loss percentages (n=5) in the different cochlear segments (half-coils) in guinea pigs exposed to pure-tone 1 33 kHz for 6 hours and with varying intensities. The relation between cochlear distance and frequency localization is given in the lower left and OHC-loss percentages in the control animals in the low

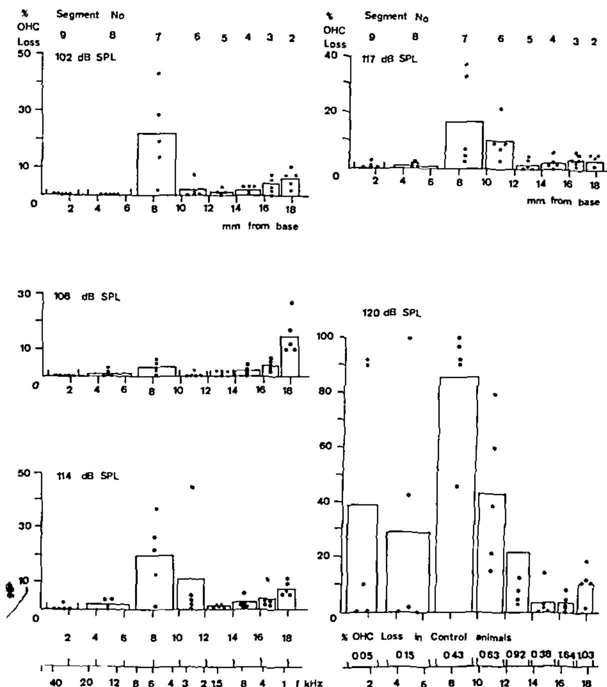


Fig 16 Mean value (columns) and individual values (dots) of OHC-loss percentages (n=5) in the different cochlear segments (half-coils) in guinea pigs exposed to pure-tone 3 85 kHz for 6 hours and varying intensities The relation between cochlear distance and frequency localization is given in the lower left and OHC-loss percentages in the control animals in the lower right

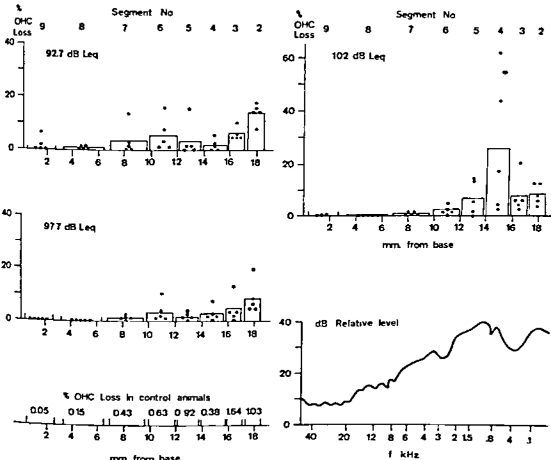


Fig. 17. Mean value (columns) and individual values (dots) of OHC-loss percentages (n=5) in the different cochlear segments (half-coils) in guinea pigs exposed to impulse sound for 6 hours and varying equivalent continuous sound level, L_{eq}. OHC-loss percentages are given for the control animals in the lower left. The lower right diagram shows the FFT-analysis of the impulse sound converted to the frequency response scale of the cochlea

was a rather small average damage of about 4 % at 92 7 and 97 7 dB L_{eq} This damage clearly exceeded that in the controls ($p < 0 01$) At 102 dB L_{eq} the damage in segment 4 was 26 % (ranoe 3-63 %) In fio 17 the frequency spectrum distribution of the impulse sound from fig 4 is adapted to the cochlear frequency scale The damage center, in segment 4, coincided fairly well with the maximum energy of the frequency spectrogram of the impulse

When the damage values of the animals in series III at 102 dB L_{eq} were compared to those of the animals exposed to a pure-tone of equal duration and corresponding intensity (6 hours, 102 dB SPL, 1 33 kHz, fig 15) the damaoe was slightly larger in the impulse animals A wider spread of the damage in segment 4 of the impulse-exposed animals (range 3-63 %) than in the 5th segment of the pure-tone animals (range 3-11 %) could indicate a more injurious effect of the impulse noise ($p = 0 11$)

Figure 18 shows a number of diagrams illustrating series IV, where the equivalent continuous sound level, L_{eq} was kept constant at 102 dB, while the exposure time was varied The distribution of the hair cell damaoe was about the same in the 6- and 12-hour experiments, but spread to seqments 5 and 6 (higher frequencies) and to segment 3 (lower frequencies) in the 24-hour experiments In the 48-hour experiment further extension of the damaoe was seen mainly in the basal (higher frequency) direction, whereas a reduction was observed in segment 2 The basal 6 mm (frequency region 8-50 kHz) was not damaged All the other segments in these animals were damaged more than the corresponding segments in the controls Here, too the localization of the damage was fairly consistent with the freouency spectrum

3 2 3 *Total damage pattern*

3 2 3 1 *Pure-tone series* The percentages of the total number of OHC damaged in segments 2-8 was calculated and is given in fig 19 as a function of sound intensity for both pure-tone series The values for all separate guinea pigs are also given and are clearly separated from those in the controls A characteristic of both series was that the total damage was almost constant from 102 to 114 dB SPL and then sharply increased at 117 and 120 dB SPL There seemed to be a damage threshold somewhere between 114 and 117 dB SPL for 1 33 kHz and between 117 and 120 dB SPL for 3 85 kHz Before the threshold was reached the damage in the two series was comparable, 2-10 %, but above the threshold the damage was twice as large for 3 85 kHz as for 1 33 kHz at 120 dB SPL From figs 15 and 16 it is quite clear that the damaoe of the apical segments 2 and 3 did not increase with the intensity

3 2 3 2 *Impulse series* Figure 20 gives the percentage of the total OHC-damage in segments 2-8 in the animals of series III The fiqure shows that

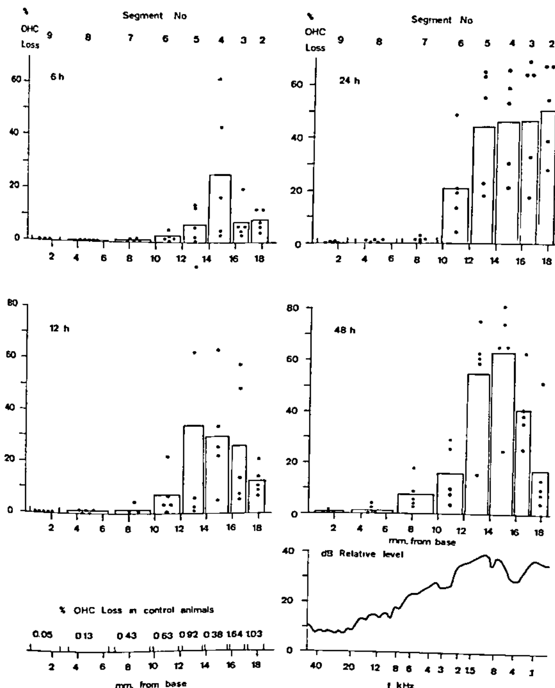


Fig 18 Mean value (columns) and individual values (dots) of OHC-loss percentages (n=5) in the different cochlear segments (half-coils) in guinea pigs exposed to different durations of impulse noise at the equivalent sound level 102 dB The OHC-loss percentages in the control animals are given in the lower left The lower right diagram gives FFT-analysis of the impulse sound converted to the frequency response scale of the cochlea

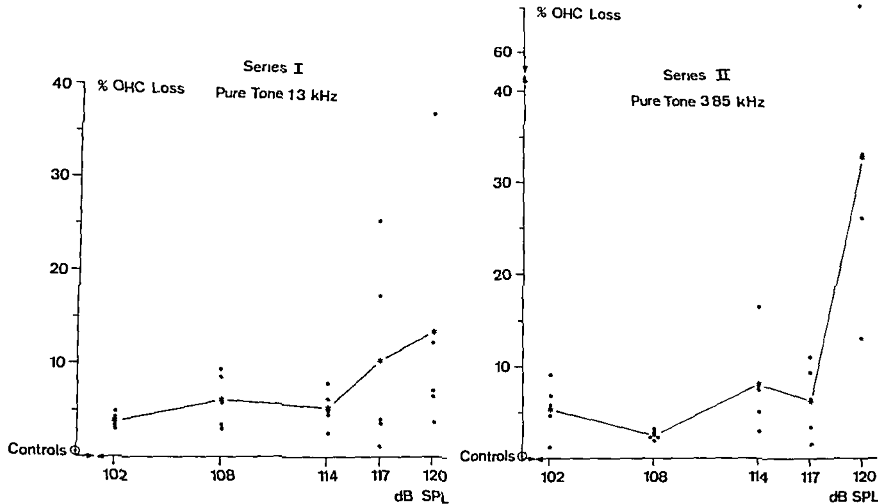


Fig 19 Mean value (asterisks) and individual values (dots) of the total OHC-loss percentages in segments 2-8(3 1/2 coils) for groups of guinea pigs (n-5) exposed to a pure-tone for 6 hours as a function of the sound pressure level(SPL) I Exposure to pure-tone 1.33 kHz. II Exposure to pure-tone 3 85 kHz

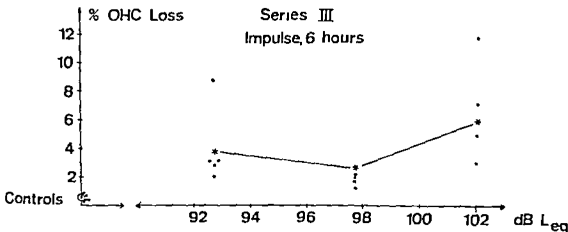


Fig 20 Mean value (asterisks) and individual values (dots) of the total OHC-loss percentages in segments 2-8 for 3 groups of guinea pigs (n 5) exposed to repetitive impulse noise for 6 hours The equivalent sound level was varied by changing the repetition rate of the impulse between the different groups of animals The OHC-loss percentages for the control animals are plotted on the ordinate

the damage was rather small, yet clearly exceeding that in the controls The damage did not vary to any significant extent with the intensity of the exposure

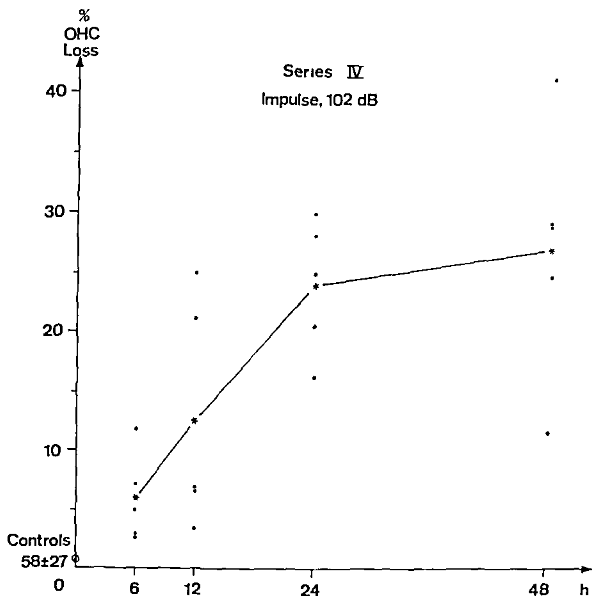
Figure 21 gives the results in series IV The first value at 6 hours was the same exposure as the last value of 102 dB L_{eq} in fig 20 There the 102 dB L_{eq}-value was maintained but the exposure time was doubled in each exposure The mean values of the 6 h, 12h and 24 h exposures give an almost straight line with a doubling of the damage for each time doubling This trend is broken for the last point at 48 h The curve seems to level off with only a slight further rise

4 DISCUSSION

4 1 METHODOLOGICAL CONSIDERATIONS

4 1 1 *Acoustics*

The choice of acoustic equipment and parameters was determined by the desire to achieve maximal stability of prolonged exposures Many authors do not describe the acoustic properties of their devices or of their stimuli, for which reason it is difficult to make comparisons with these experiments In noise studies, where longer exposures are preferred, the animals must be able to move about freely, so that they can feed and avoid influences of stress To achieve this, a cage was used in the present investigation, which resembles constructions in other experiments (Davis et al, 1935, Luz et al, 1973, Blakeslee et al, 1978, Clark & Bohne, 1978) Spoendlin (1976) used a la



'ig 21 Mean value (asterisks) and individual values (dots) of total OHC-
oss percentages in segments 2-8 for 4 groups of guinea pigs (n=5) exposed
to a repetitive impulse sound of the same equivalent sound level. The exposure
duration was varied between the different groups of animals The values in
the controls are given on the ordinate

metal container whose acoustic properties were not described

The disadvantage of letting the animals move about in the cage is that they may screen each other, but as their positions in the cage was found to be randomized during the fairly long exposures they were assumed to have been equally exposed Besides the possibility of screening effects, there is also that of amplifying effects and these may be one of the reasons for the spread of the damage, particularly in the animals exposed to the higher intensities The difference between the sound pressures at the eardrum and at the microphone position can to some extent be estimated from Sinyor & Laszlo (1973) This would mean a pressure difference of about + 3 dB at 1 33 kHz and - 3dB at 3 85 kHz between the sound pressure at the eardrum and that measured by the microphone For the impulse sound from the hammer the difference would not exceed ± 5 dB at any frequency

4 1 2 *Experimental series*

For each of the 17 exposures 5 animals were used (16 noise groups and 1 control group) This made it possible to vary the noise parameters and maintain a statistical safety within the groups This differs from the work by other authors who did not use separate control animals (Blakeslee et al, 1978, Henderson et al, 1979) or consisted of single or few animals (Poche et al, 1969) or without description of the statistical analysis of the animals (Spoendlin, 1971)

4 1 3 *Animals*

Female, dark-eyed guinea pigs from an old wellbred stock without hereditary defects or signs of disease were used to avoid differences in sensibility to noise caused by any variation with sex or melanin content (Hood et al, 1976, Ulehlová & Voldrich, 1978, Carter, 1980) In none of the 85 animals (170) ears did preparation of the ears reveal signs of existing or earlier otitis media, which otherwise is a serious problem in some strains (Stockwell et al, 1969)

The Preyer reflex, which was elicited by an audiometer headset, was used only for primary selection of the animals before the experiment Ernstsson (1972) concluded that owing to recruitment the Preyer reflex is sometimes useless as a test of acuity and therefore the findings after exposure were not evaluated

4 1 4 *[illegible] analysis*

The evaluation of severely noise-damaged areas and their distinction from preparation damage of the specimens was done in accordance with Stockwell et

tively 1 33 kHz and 3 85 kHz pure-tone stimuli were used OHC-damage apical to the stimulus frequency was also observed by Stockwell et al (1969) who discussed subharmonics as a possible source of this pattern

Both series at pure-tones showed damage in segments 2 and 3 Unlike the damage in the other segments, the OHC-losses in the apical coil seemed to vary independently of the stimulus intensity The damage thus seemed to be related to the occurrence of noise, but not to its intensity The reason is unclear A possible explanation is given by Axelsson (1974), according to whom the apical region of the guinea pig cochlea has a simplified vascular system allowing fewer possibilities of adjustment to variation of the circulation This possibility is emphasized by Bernstein & Schuknecht (1967), who found apical hair cell losses in the guinea pig after temporary obstruction of the labyrinthine arteries The variation of the damage in segments 2 and 3 did not affect the total, calculated OHC-loss given in fig 19 for which reason they have been included in the calculation

The existence of a threshold or critical level, which in this 6-hour experiment was found between 114 dB and 117 dB SPL for 1 33 kHz and between 117 and 120 dB SPL for 3 85 kHz, has been described also by other authors (Ruedi, 1954, Ward & Nelson, 1971, Spoendlin & Brun, 1973) A general trend of both curves in fig 19 is that the spread of the individual total damage increased with the intensity of the sound These patterns could be in agreement with the theory that there is a sound level below which metabolic effects are predominant and above which the mechanical effects are dominating

It is evident from the results of Spoendlin & Brun (1973) that if the exposure time is decreased, the critical level occurs at a higher sound intensity The critical levels from their investigation and the level found in the present work are shown in fig 22 There are, of course, uncertainties in the determination of the positions of the critical levels, and the frequency content of the stimuli is not the same, but nevertheless it is possible to combine the points with a line, which seems to comply with the equal-energy principle

The total energy principle, which states that the damage to hearing is proportional to the total energy, is hard to adapt to the different exposures of series I and II and to combine the whole sound intensity range with the occurrence of the OHC damage using the same proportionality constant It could be done for series I but not for series II, underlining the hypothesis that when the sound intensity is increased, the cause of the OHC-damage shifts from mainly metabolic exhaustion to mechanical stress (Spoendlin & Brun, 1973, Price, 1979)

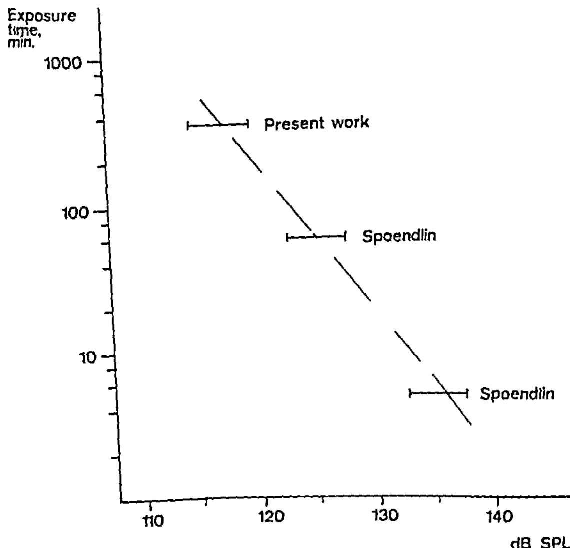


Fig 22 Comparison between the present results and the results of Spoendlin & Brun (1973) In the figure the value of the critical level of intensity ± 3 dB is plotted as a function of exposure duration for the guinea pig.

Above the critical level there is a difference in the severity of the damage between the animals exposed to 120 dB SPL at 1.33 kHz and those exposed to 120 dB SPL at 3 85 kHz This difference in damage does not seem to be related to omission of A-weighting, differences in acoustic properties of the anechoic chamber, ear canal resonance or middle ear impedance (Sinyor & Laszlo, 1973, Manley & Johnstone, 1974) The difference might indicate a more damaging effect of the 3 85 kHz tone under similar stimulus conditions and is in agreement with the results of Stockwell et al (1969), where there was a clear difference in the damage caused by exposures to 4 kHz and to 1 k

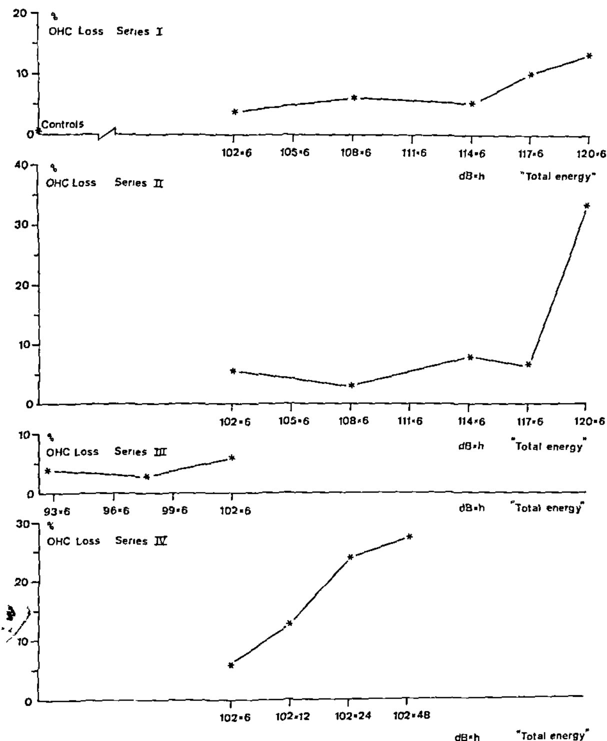


Fig. 23. Mean values of total OHC-loss percentages for each group of guinea pigs plotted as a function of the "total energy".

upper value, which for the chosen sound variables was 24 hours For longer durations the increase of damage seemed to level off to an asymptotic approach to an upper damage limit

(5) Groups of guinea pigs exposed to pure-tone of intensities below the critical level and groups of animals exposed to impulse noise of varying L_{eq} (equivalent sound level) showed damages which did not increase in proportion to the increase in total sound energy

(6) Comparison between the OHC-damages caused by a continuous pure-tone of 1 33 kHz for 6 hours at 102 dB SPL and a repetitive impulse noise with the energies centered around 1 kHz during 6 hours at 102 dB L_{eq} indicated a slightly higher mean damage in the impulse-noise exposed animals and a wider spread of the damage in the target segment of the cochleae

(7) Comparison between the OHC-damage caused by impulse noise and the damage caused by pure tone at equal total energies demonstrated so wide a difference that the equal-energy concept could not possibly be applied to this situation

(8) The experimental material in this investigation showed evidence both for and against the equal-energy concept The conclusion is therefore that a generalized equal-energy principle cannot be applied throughout the whole range of the different parameters used in this investigation

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SUPPLEMENT 368

Elevated Stapedius Reflex Threshold and Pathologic Reflex Decay

Clinical occurrence and significance

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SUPPLEMENT 368

Elevated Stapedius Reflex Threshold and Pathologic Reflex Decay

Clinical occurrence and significance

BY

ANITA HIRSCH and HENRY ANDERSON

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This study was supported by grants from Stiftelsen Tysta skolan and The Carl-Axel Hamberger Fund

STOCKHOLM [illegible]

INTRODUCTION

Although the activity of the middle ear muscles in man was subjected to extensive physiologic studies already during the last century, the idea of utilizing the reflex activity in these muscles in the diagnosis of hearing impairment seems first to have been suggested by Kobrak (1948) However, the early methods for recording activity in these muscles were not particularly suited to clinical use With the introduction of acoustic impedance measurement of the ear, new fields were opened and Metz (1946, 1952) was first to report results in clinical series of cases examined by this method Although his work mainly concerned the acoustic properties of eardrum and ossicular chain, he reported some findings on the middle ear muscle reflexes in ears with sensorineural hearing loss (Metz, 1952) observations that became the foundation for a new school in the diagnosis of hearing impairment However, this new method of investigation did not break through until the mechanical acoustical bridges initially used, had been replaced by more expedient electroacoustic equipment

Metz (1952), when stimulating the affected ear in two cases of acoustic tumours found no recordable middle ear muscle responses by recording from the opposite ear, his finding was verified by similar observations by Kristensen & Jepsen (1952), and in other disorders of suspected retrocochlear origin by Thomsen (1955) However, with the methods then existing, the result had to be interpreted with some reservation as an absence of reflex responses could not with certainty be ascribed to the sensorineural damage, as also a minor conductive defect in the opposite recording ear would give the same result, a fact that early was recognized With the further development of the technique for clinical impedance measurement, this source of error has been eliminated Prerequisites for reflex recording such as the condition and position of eardrum is established by tympanometric air pressure loading and by checking the motoric activity of the muscles and the mobility of ossicular chain by non acoustic elicitation of reflex activity (Klockhoff & Anderson, 1959, Djupesland, 1967), in later years by ipsilateral reflex measurement

The early observation on the behaviour of the stapedius reflex threshold in retrocochlear hearing impairments during the years have been confirmed in extended materials of cerebello-pontine angle tumours (mainly acoustic tumours) by Anderson et al (1970), Lidén & Korsan Bengtsen (1973), Jerger et al (1974), King et al (1976) and Johnson (1979) Pathologic reflex threshold elevation also has been described in retrocochlear lesions of other origin than the cerebello-pontine angle tumours, such as in brainstem lesions (Greisen & Rasmussen, 1970), in athetosis (Lidén 1970) and in multiple sclerosis (Coletti, 1975)

Another stapedius reflex response parameter that has been studied from the diagnostic point of view is the decline in reflex response amplitude on prolonged acoustic stimulation the stapedius reflex decay (Anderson, 1969) That such a phenomenon existed was early known from physiological experiments in animals ('Reflextaubheit', Kato, 1913) and that the stapedius reflex behaved similarily in humans was early confirmed by otoscopic examination (Luscher, 1929, 1930) and later by acoustic impedance measurement (Gjaevenes & Sohoel, 1966, Djupesland et al , 1967) An attempt to correlate the degree of stapedius reflex decay to the individual susceptibility to noise induced hearing loss was made by Johansson et al (1967)

A clinical diagnostic approach with respect to the stapedius reflex decay was first reported by Anderson et al (1969) who after studying a material of cases with eighth nerve dysfunction concluded that a pathologically rapid reflex decay can serve as a sensitive indication of such lesions The same authors suggested a standardized procedure for reflex decay measurement and formulated a criterion for the classification of pathological degree of reflex decay In this study evidence is also given that the origin of the pathologic forms of reflex decay, similar to that occuring normally, was to be found in the afferent branch of the reflex arc, and probably caused by damage to the eighth nerve structure

This 'sensory' reflex decay must be kept strictly apart from such forms of reflex decay that may occur in neuromuscular disorders, such as myastenia gravis (Blom & Zakrisson, 1974) A differentiation with respect to sensory or neuromuscular (motoric) origin of the decay is possible by use of the two-tone technique described by Gjaevenes & Sohoel (1966)

Anderson's et al (1970) opinion on reflex decay as a valuable test for identifying retrocochlear hearing impairments has been confirmed by a number of clinical series (Jerger et al , 1974, King et al , 1976, Sheehy & Inzer, 1976, Johnson 1979) as is also the high sensitivity of the reflex decay test in other forms of retrocochlear disorders (Coletti, 1975, Hall 1977) The claimed higher sensitivity of the reflex decay test in comparison with traditional differential diagnostic tests in the diagnosis of retrocochlear lesions has also been demonstrated by Shiffman et al (1976)

On the contrary, some authors express doubts on the reliability of the reflex decay test Chiveralls (1977), in three cases of acoustic tumours, where prerequisite conditions for reflex decay testing seem to have been present, found the test positive in only one case Further, the author complains over the excessive proportion of 'false positive' outcomes obtained with the test Terkildsen el al (1977) report two cases of verified acoustic tumours with moderate hearing loss where stapedius reflex testing showed normal results, both with respect to reflex threshold and reflex decay

Thus, with some exceptions, the presence of stapedius reflex threshold elevation and pathologic reflex decay have been accepted as indications of a retrocochlear lesion However direct comparison of the results of reflex measurements obtained by different authors is difficult, due to differences in recording technique and criteria for stapedius reflex response pathology Comparison to other studies is further complicated by the fact that the criterion of pathology for

reflex decay initially set by Anderson et al (1969) was strictly tied to cases with an average hearing loss of 60 dB or less a fact that seems to have been overlooked in most comparable studies The way results generally have been presented in these studies gives little or no opportunity to survey the occurrence of 'false positive' cases (with respect to cerebello-pontine angle tumours) obtained with the reflex tests This question must be regarded as of vital interest for an estimate of the value of both the reflex threshold test and the reflex decay test in clinical practice

The object of the present study is to evaluate in a material of cases with insignificant to moderate sensorineural hearing impairments the diagnostic capacities of the reflex threshold test and the reflex decay test evaluated in relation to some traditional differential diagnostic hearing tests Further, the aim is to establish the probability of an expansive process affecting the eighth nerve in cases of a positive outcome of reflex tests and finally, to discuss whether an adjustment of the initially suggested criterion for reflex decay pathology can be regarded as motivated

TECHNIQUE

Equipment

All audiometers used in this study have been calibrated *according to ISO Recommendation R 389 (1964)* and checked to fulfil the technical demands set up in IEC Standard No 177 (1965) – For masking in the AC/BC tests, band filtered white noise was used The characteristics of the noise bands and calibration of noise levels were those described by Lidén et al (1959)

The tone pairs for Fowler's test (ABLB) were controlled by a gating unit connected to the audiometer Tones of 1 s duration were delivered to each ear, separated by a silent interval of 0 25 s The tone pair could be started at right or left ear The rise and decay times of the tone pulses were 50 ms

Tone decay was graphically recorded by a method designed by Anderson (1960) and routinely used in the laboratory for nearly 20 years (Fig 1) The technique requires some modifications of the Békésy audiometers The circuit for patient response button could be locked by a switch so that depressing of the response button did not cause the traditional decrease in test tone intensity but instead locked the attenuator at the intensity present when the response button was activated Release of the response button started – as in normal Békésy audiometry – an increase in the test tone intensity The basic idea behind this mode of tone threshold decay recording is to submit the ear to the constant near threshold stimulus required for the threshold decay to manifest itself distinctly

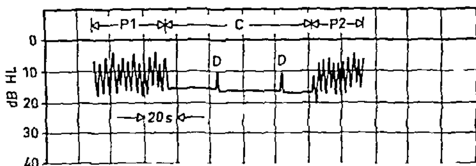
The technique used for tympanometry and reflex recording in this study is essentially that described by Klockhoff & Anderson (1959), Klockhoff (1961) and Anderson (1969) although some technical improvements have been made during the years

The carrier[1] tone is fed through a flexible silicone rubber tube to one of the two channels of the ear probe and the resulting sound pressure in the meatus is recorded by a microphone connected to the other probe channel by a similar rubber tube Both transducer and microphone are virtually insensitive to the tympanometric air pressure changes The picked up signal is filtered and amplified and digitally displayed in linear units as a measure of the steady state acoustic reflection from the ear, whereas *changes* in the acoustic reflection, caused by eardrum air pressure loading or middle ear muscle contractions, are recorded in dB on a logarithmic strip chart recorder When ipsilateral reflex recordings are performed, the original two-channel ear probe is replaced by a probe having three channels To the third channel is directly connected a miniature earphone *which delivers the stimulus tone*

The rubber tubes connecting the carrier tone transducer and microphone to the probe, act as a primary acoustic filter with a resonance frequency mainly determined by the length of the tubes (2 x 32 cm, inner diameter 4 mm) The electrical

[1] The expression carrier is preferred to probe as the latter denotation in combinations such as probe tone probe ear etc becomes somewhat ambiguous in the case of ipsilateral reflex measurements

EXAMPLE A



EXAMPLE B

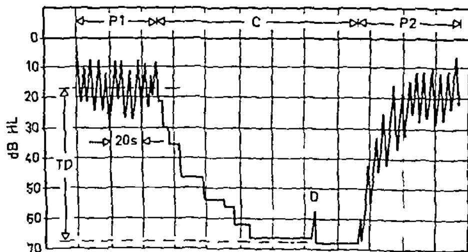


Fig 1 Examples of recordings obtained with the method used for tone decay test Test starts with fixed frequency standard pulsed tone presentation and conventional Békésy recording technique (P 1) until a reliable HL reference can be determined The rate of amplitude change is set at 5 dB per s During recording period C the tone is switched to continuous presentation and the reverse action of the attenuator is locked as [illegible] the test is [illegible] the C [illegible] its in a [illegible] amount of tone decay present [illegible] the return action of [illegible] oints (D) gives a good [illegible] e B P 2) ensures that

filtering of the carrier tone is achieved by a band pass filter with a bandwidth of 1 per cent and a slope of 70 dB per octave

The system works within a carrier frequency range of approximately 500 to 800 Hz The nominal frequency at which measurements generally are started is 550 Hz, occasionally abnormal middle ear conditions may require adjustment to a different carrier frequency to obtain satisfactory recordings

This method of tympanometry does not aim at measuring the acoustic impedance of the ear or such functions as the equivalent volume, nor at separating the impedance in its resistive or reactive components Instead, all values relating to the steady state reflection from the eardrum are given as per cent reflection, where 100 per cent reflection is the value obtained with the probe connected *to a 1 5 cc hard wall test cavity*

The steady state reflection values for normal ears lies between 13 4–34 7 per cent * The change in reflection caused by tympanometric pressure loading of the eardrum is recorded as dB deviation from the steady state reflection value The amplitude change due to negative or positive pressure loading of the eardrum (10 mm Hg = 136 mm H_2O) ranges in normal ears between 4 2 and 6 8 dB * Values for both reflection percentage and tympanometric pressure response apply to the nominal carrier tone frequency of 550 Hz The change in reflection resulting from activation of the middle ear muscles is likewise recorded as dB deviation from steady state value The stapedius reflex response amplitude, measured in normal ears with a carrier tone of 550 Hz and elicited by contralateral stimulation with 1000 Hz at a level 10 dB above individual reflex threshold, range between 0 9 and 1 9 dB *

In the setting used for clinical routine work, the over all recording speed of the system – acoustic filter, electronic filter and recorder included – is 55 dB per second, a figure that clinical experience has found to prove a good compromise *between freedom from disturbances and recording speed required with respect to* the physiological events to be recorded If necessary, the recording speed can be increased to 100 dB per second at the cost of some reduction in the signal-to-noise ratio The recording onset delay, measured from start of sound pressure change in carrier tone to first deflection of recording pen is in setting for normal use 12 ms but can, if required, be decreased to 4 ms

Much attention has been paid to the design of the air pressure device which supplies the over and underpressures required not only for the tympanometric measurements but frequently, too, in the reflex recordings In contrast to the continuous and rather slow change in air pressure commonly used in clinical tympanometry (but seldom stated with respect to time course), the method *arrived at here uses well defined air pressure pulses – negative and positive –* with a rise time of 3 seconds and a reset time of 0 2 seconds and with negligible non linearity in the pressure *vs* time course This mode of pressure loading has been denoted 'transient pressure course' to distinguish it from the more common 'sweep pressure course' (Anderson 1977, Tyler 1979)

Another feature of the pressure device is that it can be set to maintain with

* $p^{25} - p^{75}$ range

high accuracy any pressure within the working range of the instrument – positive or negative – over an infinite period of time This pressure holding system is designed to keep the pressure constant within ± 1 mm H_2O of the set value The digital-controlled servo-assisted pump mechanism automatically compensates for minor leaks between probe and meatus If the leak is large enough to act as an acoustic shunt and thereby introduce inaccuracies into the measurements (leakage more than 1 5 ml/min), a warning signal is given

Comments on the technique for tympanometry and reflex measurements As in the audiometric testings, the tympanometry and reflex measurements were performed with the patient seated in a sound insulated chamber separated from operator and equipment This arrangement was used as it has been demonstrated that external stimuli of various kinds, due to influence from the CNS, can cause considerable variations in the recorded reflex responses (Klockhoff, 1961)

The choice of a carrier frequency in the vicinity of the resonant frequency of the ear, combined with the acoustic pre-filtering of the rubber tube arrangement, contributes to make the recording unusually free from disturbances This specific feature has several advantages First, the carrier tone intensity can be kept as low as 60 dB HL (66 5 dB SPL as measured in a 2 cc IEC coupler) a level sufficiently low to preclude the risk of cross-potentiation with the stimulus tone (*cf* 'Discussion') Second, the flexibility of the tubes allows for reasonable head movements without the introduction of disturbances of importance in the recording – an indispensable requirement, for instance, when using the air-jet stimulation to elicit middle ear muscle activity And third, the insensitivity to disturbances is vital for the proper recording of responses to non-acoustic stimulation where mechanical noise is likely to occur, such as when scratching the patient's cheek with a pin or when the patient is urged to close the eyes excessively tight, a manoeuvre inevitably accompained by other movements of head and facial muscles

With respect to the procedure used for tympanometry, the transient air pressure technique was designed to avoid the adverse effects resulting from prolonged pressure loading of the delicate structures of the eardrum and middle ear mechanism The transient pressure technique exerts no more strain on the eardrum than what is actually necessary to obtain the required information concerning the middle ear condition It is, for instance, easy to demonstrate that exposure of an ear to prolonged negative tympanometric pressure not seldom causes a slight leakage of air through the Eustachian tube and when the tympanometric pressure slowly passes the ambient pressure level on its course from negative to positive air pressure, the resulting pattern will indicate that a positive pressure has arisen in the cavum The same type of artefact, although often more pronounced, is encountered if the patient when experiencing the sensation of pressure in the ear, instinctively reacts by swallowing Apart from avoiding such sources of error the transient technique has the advantage of always starting the pressure courses exactly from the actual ambient air pressure level and does not depend on more or less accurate indirect means of reference to the important ambient pressure level

The requirement set for the pressure device in the 'pressure hold' mode of

operation may seem excessive and requires some explanation An extremely thin or flaccid eardrum acts as an acoustic short circuit and results in abnormally low impedance which totally can shunt the minimal impedance change introduced by stapedius muscle contraction This situation is met by introducing a slight nega tive tympanometric pressure to stretch the eardrum, thereby increasing the reflection to a point where acoustic conditions permit reflexes to be recorded In a case of flaccid eardrum, the change in reflection as a function of applied air pressure follows an extremely steep course and slopes of 20 dB per 100 mm H_2O can easi ly be encountered To keep the recording baseline sufficiently stable to permit reflex recording under such conditions requires the air pressure device to main tain the set pressure with ± 1 mmH_2O

Methods criteria

Tone threshold for AC and BC were recorded by octave audiometry in most cases also supplemented by standard pulsed Békésy-audiometry In some cases ques tion arose as to whether the hearing could be regarded as normal for age Tone thresholds better than the median values for corresponding sex and age group in WSFHS (Glorig el al , 1957) were accepted as normal

Speech discrimination test Phonetically balanced Swedish monosyllabic words according to Lidén (1954) were used as test material Fifty words were presented at each level tested and results given as maximum per cent correctly repeated words Test results were evaluated empirically against shape of tone threshold curve

Fowler s test Tone pulses of 1 s duration were presented to each ear separated by an interval of 0 25 s The order of presentation right/left was randomized and the patient was instructed to assess loudness after each pair The HL of the tone pulse to the affected side was kept constant and the level of pulses to the control ear varied until loudness balance was obtained The procedure was repeated on at least two levels for each frequency tested The hearing impairment was judged to be non recruiting if an imbalance of 15 dB or more, calculated as the mean of the imbalance at 0 5 1 0 and 2 0 kHz) could be demonstrated in the HL range 80–120 dB

Tone decay was graphically recorded according to the method described under Equipment The outcome was judged as pathologic if the tone decay, determined as described in Fig 1 exceeded any of the following values 25 dB at 2000 Hz 15 dB at 1000 Hz and 10 dB at 500 Hz

Tympanometry was performed with the transient pressure technique described under Equipment The procedure ensured that the eardrums bilaterally were in a suitable condition for the stapedius reflex test

Stapedius reflex measurements were carried out by contralateral stimulation with the standard octave and half octave test frequencies 0 25 – 4 kHz As reflex thresholds were recorded the lowest hearing level (in 5 dB steps) where repeatable responses were seen in the recording If required, tympanometric air pressure was applied to equalize air pressure in meatus to that in the middle

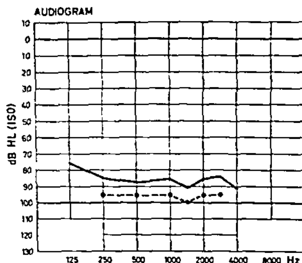


Fig 2 Normal stapedius reflex threshold (continuous curve) and limit (dashed) on and above which the individual reflex threshold value is regarded as pathologically high. For an ear to be classified as abnormal with respect to reflex threshold elevation the reflex threshold should reach the limit curve at 4 of the 6 test frequencies (after Anderson & Wedenberg 1968)

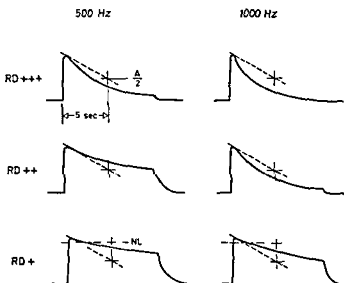


Fig 3 Schematic representation of reflex patterns used in the classification. $\frac{A}{2}$ indicate points of 50 per cent reduction of response amplitude read 5 s after onset of the 10 s long stimulus. The 10 per cent decline in response amplitude accepted as normal indicated by dash-dotted line (NL)

ear cavity or in order to achieve appropriate stiffness of eardrum to permit reflex recording (*cf* page 8) As pathologic limit for the reflex threshold was used the criterion set by Anderson & Wedenberg (1968) (Fig 2)

The absence of stapedius response from the *recording* ear was not accepted as caused by a disturbance of the afferent arc of the stimulus ear, unless sta pedius activity was demonstrated by non acoustic stimulation

Reflex decay was determined at a stimulus level of 10 dB above the individual reflex threshold by presenting the tone stimulus for 10 s at 0 5, 1 0 and 2 0 kHz *with 10 s interval between stimuli Pathologic reflex decay (RD+++) was judged* to be present if the response amplitude (logarithmically recorded) was found to decline more than 50% in 5 s on 0 5 and 1 0 kHz If more than 50% reflex decay was recorded for 1 kHz stimulation but not for 0 5 kHz, the reflex decay was classified as RD++ If decay was clearly visible in the recording but did not meet the 50% criterion for 0 5 or 1 0 kHz, the reflex decay was classified as RD+ Models of the different reflex patterns are shown in Fig 3 In this study RD++ and RD+ were not regarded to fulfil the criterion of pathology On *suspicion of motoric origin for the reflex decay this was checked by tactile* elicitation and/or two-tone test (Gjaevenes & Sohoel, 1966)

MATERIAL

The basic data for this study were collected from the laboratory records of the Department of Audiology, Karolinska Hospital The case material from which these data were obtained are patients referred for thorough audiological examination of specific otoneurologic question or patients where routine ENT examination including audiometry aroused suspicion of a retrocochlear process This category of cases is submitted to a battery of differential-diagnostic audiological tests suited to the actual diagnostic problem and the condition of the patient

Case material A consists of all such cases that were examined during 1972–75 inclusive and who demonstrated a hearing loss ≤ 60 dB (calculated as mean of HL at test frequencies 0 5, 1 0 and 2 0 kHz) and where middle ear conditions permitted application of stapedius reflex measurement The number of such cases totalled 564 of which 454 showed normal reflex thresholds and 110 cases recorded pathologically elevated reflex thresholds in one or both ears These 110 cases from case material A were submitted to analysis in this paper, 69 of the cases were men and 41 women, mean age 42 and 48 years, respectively

Case material B consists of all cases of same category that during 1969–78 inclusive were referred to the laboratory for the same reason as the cases in material A and who fulfilled the same criteria with respect to hearing function stated above Consequently, material A constitutes a part of material B The total number of cases in material B is 1366 Fortyone of these cases demonstrated pathologic reflex decay (RD+++), in 3 cases bilaterally These 41 cases from material B were submitted to further study, 19 of the cases were men and 22 women, mean age 39 and 43 years, respectively

Aetiological grouping In the treatment of results of case material A and B the following abbreviations are used

CPA = cases of cerebello-pontine angle tumours verified by radiological examination and/or surgery (Hirsch et al, 1979, Hirsch & Anderson 1980) The majority of cases in this category are acoustic neurinomas but includes other cerebello-pontine angle tumours presenting as acoustic neurinomas,

MS = cases of multiple sclerosis diagnosis confirmed by a neurologist,

BS = cases with various symptoms indicating less specific lesions to the brainstem diagnosis confirmed by a neurologist,

SD = cases with abrupt onset of deafness where audiological and medical examinations did not reveal any probable cause and where the only distinctive characteristic of the hearing impairment had been the extreme sudden onset,

Unknown = cases with hearing impairment where audiological and medical examinations did not reveal any probable cause

RESULTS

Reflex threshold elevation

The analysis of the results in the group of cases selected from material A as fulfilling the requirement of HL ≤ 60 dB in combination with pathologically elevated reflex threshold, are presented in Tables I and II The number of such cases amounts to 110, corresponding to 19 5 per cent of the total case material A of 564 patients The compilation given in Table I shows the 110 cases grouped with respect to aetiology In 32 cases, elevation of reflex thresholds was found bilaterally However, in the analysis of the results of the various differential diagnostic hearing tests given in Table II only the results from the suspected ear were used as base for calculations Among the 110 ears studied are 17 where the reflex threshold, despite average moderate hearing loss, could not be reached at all even at maximum stimulus intensity (*cf* page 10) No less than 14 of these 17 cases belonged to the CPA group which contains 20 acoustic tumours and 3 other CPA tumours affecting the eighth nerve The 3 cases with bilaterally elevated reflex thresholds in the CPA group are all cases of acoustic neurinomas and the bilateral elevation seems rather surprising with regard to the cause of lesion, a circumstance that will be discussed further Both in the SD and Unknown groups, the proportion of bilaterally elevated reflex thresholds is remarkably higher than in the other groups, a circumstance probably to be explained by an over-representation of cases with hereditary hearing defects (Anderson & Wedenberg, 1968)

Reflex threshold pattern In the majority of cases, the pathologically elevated reflex threshold showed a slope towards higher frequencies This pattern dominated in all cause groups In only 15 of the 142 ears in Table I with pathologic reflex thresholds, was an inversed pattern found, i e , the elevation of the reflex threshold reached its maximum in the lowest frequency range With respect to cause grouping, these 15 ears in 13 patients were classified as follows 2 cases of brainstem leison, 2 cases of sudden deafness and 7 cases belonging to the Unknown category Further, this uncommon reflex threshold pattern was found *in the opposite ear* in 2 cases of acoustic tumours In one of these cases, the ear affected by the tumour showed a total absence of recordable reflexes and in the other case, the reflex threshold was reached at maximum HL on the middle frequencies only

Elevated reflex thresholds versus results of psychoacoustic tests The outcomes of the traditional differential diagnostic tests in the 110 cases are shown in Table II, which also contains information on mean hearing level and mean age for the different aetiological groups

With regard to average hearing level, the only group that deviates considerably is the BS cases, which demonstrates markedly lesser average hearing loss than the other groups Nor is the difference in mean age between groups significant, the MS group here being a striking exception which coincides with the known early age of onset of this disease The outcomes of the various psycho-acoustic tests

Table I *Aetiological classification of cases (ears within brackets) with pathologic reflex threshold elevation in Material A (N = 564) Abbreviations CPA = cere bello pontine angle tumour MS = multiple sclerosis BS = brainstem lesion SD = sudden deafness For further explanation see Material*

	Aetiology					
	CPA	MS	BS	SD	Unknown	Total
Cases	23	4	13	21	49	110
(Ears)	(26)	(5)	(16)	(27)	(68)	(142)

Table II *Results of the differential diagnostic tests in the 110 cases from Table I with pathologically elevated stapedius reflex thresholds related to aetiology Number of test results indicating a retrocochlear lesion is here calculated as per cent of total number of cases (in brackets) tested in each group For explanation of abbreviations see Table I*

	Aetiology				
	CPA	MS	BS	SD	Unknown
Mean age	46 4	27 3	43 5	40 4	46 8
Mean HL dB	32 6	34 0	17 0	40 8	30 0
Speech discr	45 % (22)	33 % (3)	11 % (9)	44 % (18)	17 % (35)
Tone decay	83 % (23)	50 % (4)	10 % (10)	7 % (14)	11 % (46)
Fowler s test	67 % (21)	67 % (3)	0 % (6)	9 % (11)	20 % (15)
No of cases	23	4	13	21	49

show a distinct dominance of positive results in the CPA group and in the MS group In the remaining aetiological groups, the only percentage figure that approaches those of the CPA group is the result of speech tests in the SD group

The conclusion is, thus, that in the two groups with confirmed or highly probable lesion to the eighth nerve, a connection seems to exist between an elevation of reflex threshold and of positive outcome of the psycho-acoustic tests and that this connection is less pronounced in the brainstem lesions and in the less well definable pathologies that constitute the SD and Unknown groups

Reflex decay

In the study of occurrence of reflex decay, case material B consisting of 1366 cases was used The analysis was confined to the 41 cases (=3%) which demon strated a hearing loss ≤ 60 dB in combination with pathologic reflex decay, according to the existing criterion (RD+++) and the result, classified according to diagnosis is shown in Table III The majority of the 41 cases belong to the groups with diseases known to affect the eighth nerve In 37 of the 44 ears, pathologic reflex decay was present in combination with elevated reflex threshold In the remaining 7 ears RD+++ was the only abnormality in the recordings

Reflex decay versus results of psycho-acustic tests Table IV shows the outcomes of the differential diagnostic tests in the ears with pathologic reflex decay As was the case in the reflex threshold study, the MS group deviates clearly from other groups with respect to mean age and mean HL However, in the selection made according to reflex decay, a distinct reduction of proportion of positive outcomes of the psycho acoustic tests is recorded for this specific group On the other hand, the proportion positive outcomes of these tests is considerably increased in the SD and Unknown groups with scores approaching those of the most well defined category of lesions, the CPA group In contrast to the selection based on reflex threshold elevation where 13 BS cases were found, no such cases appear in the RD+++ grouping

Interrelation between elevated reflex threshold and reflex decay In the foregoing, the incidence of elevated stapedius reflex thresholds and reflex decay has been treated as separate phenomenae Further, it is of interest to analyse how these two parameters appear in combination, and for this purpose material A was used Table V gives the result of this analysis and as a dominating reflex pathology pattern appears here, the group of cases with elevated reflex thresholds in combination with absence of reflex decay whereas the presence of reflex decay in combination with normal reflex threshold forms an extremely rare combination

It was also judged to be of certain interest to investigate how the two reflex parameters combine in hearing impairments of known retrocochlear localization As control material, was here chosen the CPA tumour cases as they constitute the category where a positive verification of the retrocochlear lesion is possible to obtain within reasonable period of time

The result is given in Table VI Here it has to be observed that in order to

Table III *Aetiological classification of cases (ears within brackets) with pathologic reflex decay (RD+++) in material (N = 1366)*
For explanation of abbreviations see Table I

	Aetiology				
	CPA	MS	SD	Unknown	Total
Cases	14	8	6	13	41
(Ears)	(14)	(10)	(6)	(14)	(44)

Table IV *Results of the differential diagnostic tests in the 44 ears from Table III with pathologic reflex decay related to aetiology Test results indicating a retrocochlear lesion are given as per cent of ears tested number of ears tested within brackets*
For explanation of abbreviations see Table I

	Aetiology			
	CPA	MS	SD	Unknown
Mean age	45 7	31 8	37 0	44 3
Mean HL dB	25 2	16 1	31 3	41 3
Speech discr	36 % (14)	25 % (8)	80 % (5)	45 % (11)
Tone decay	43 % (14)	20 % (10)	60 % (5)	15 % (13)
Fowler s test	55 % (11)	33 % (6)	25 % (4)	57 % (7)
No of ears	14	10	6	14

Table V *Relation between reflex threshold and reflex response characteristic in the cases of Mateiral A (N = 564)*

		Reflex threshold		
		Normal	Elevated	Not reached*
Response characteristic	Normal	79 9 %	12 1 %	–
	RD+++	0 5 %	2 0 %	–
	Not recordable	–	–	5 5 %

* Column Not reached also includes cases where reflex thresholds only were reached at or near maximum output level from audiometer and the reflex characteristic consequently could not be determined

Table VI *Incidence of CPA cases within each group of reflex parameter combinations Number of CPA cases given in relation to all cases assigned to the group in question Case material same as for Table V except for RD+++ groups where case material B was used*

		Reflex threshold		
		Normal	Elevated	Not reached*
Response characteristic	Normal	0/451 (0 %)	4/68 (5 9 %)	–
	RD+++	3/6 (50 %)	11/35 (31 4 %)	–
	Not recordable	–	–	16/31 (51 6 %)

*Column Not reached includes cases where reflex thresholds only were reached at or near maximum output level from audiometer and the reflex characteristic consequently could not be determined

obtain a satisfactory numer of cases in the study of the less common combinations (RD+++), material B was used for these groups In these case materials, it can be demonstrated that the group with a total absence of reflex responses contains the highest percentage of CPA cases The group of pathologic reflex decay, in combination with or without elevated reflex thresholds, contains the second highest proportion of CPA cases. In the group with normal reflex characteristics, no CPA case was found It is interesting to find that the group elevated reflex threshold combined with normal reflex decay only contains a small fraction of CPA cases, i e , that an elevated reflex threshold per se seems to be of low significance for the identification of CPA cases However, it is to be observed that no less than 3 of the 4 CPA cases in this specific group, although formally classified as normal, in fact demonstrated a RD++ response characteristic, a circumstance that will be further considered in the Discussion

An assay of the two reflex tests' ability to identify CPA cases must also take into consideration the fact that these processes, with very few exceptions, are unilateral A survey of material A from this point of view shows that with elevated reflex threshold as the sole parameter and with the requirement of unilaterally added, this increases the number of identified CPA tumours by only about 5 per cent, in the RD+++ selection, even less The explanation is to be found in the compilations of Table I and III which show that elevated reflex threshold and pathologic reflex decay, irrespective of cause, often are manifested as unilateral phenomenae

Conclusion of results

In a clinical case material of sensorineural hearing impairments with hearing loss ≤ 60 dB, an analysis of the results of stapedius reflex examinations has given the following results

When analyzing the material with pathologic reflex threshold elevation as the selecting parameter the study shows that not less than two thirds of the ears demonstrating this specific characteristic belonged to the aetiologically non-specific Sudden Deafness and Unknown categories (64%) whereas the more specific and more easily confirmed lesions CPA, MS and BS constituted the remaining one third of the ears

With pathologic reflex decay as the selecting parameter, the proportion of ears belonging to the non specific aetiological categories is reduced to below half, and the more specific categories of lesions increase to 55% of the total number of ears showing pathologic reflex decay

The positive results in the psycho-acoustic tests – accepted as indicating retrocochlear dysfunction – showed on average a higher connection to pathologic reflex decay than to pathologically elevated reflex threshold The most pronounced diffeence is observed in the non specific groups where the proportion of positive outcomes increases from 18 to 47 per cent in the reflex threshold study and in the reflex decay study, respectively

Judged from the result of this study, the probability of encountering normal

stapedius reflex threshold in a case of CPA tumour seems very low When the two reflex parameters are evaluated separately the results indicate that in one out of six ears (one out of five cases) with reflex threshold elevation, the cause could be attributed to a CPA tumour and that the same cause was present in one out of three ears with pathologic reflex decay

When considering the grouping in Table VI with reference to the cases who demonstrated pathologic reflex decay in combination with normal or pathologically elevated reflex threshold and included the cases where the reflex threshold was elevated above limit of audiometer (which leaves the question of possible reflex decay unanswered), it is evident that no less than 30 of the 34 CPA tumour cases in the present study fulfil either of these criteria

DISCUSSION

Method for tympanometry and reflex recording The technically straightforward principle of operation of the recording method used in this study, requiring just a moderate amount of amplification, minimizes the linearity problems frequently associated with high-gain bridge circuitry and phase dividing network Further, the recording method used here seems clearly superior to that based on indirect function such as measuring feedback control voltage, a somewhat roundabout principle of operation where recording artefacts in the form of time delay and overshoot seem hard to overcome Instead, the method described offers excellent stability and freedom from disturbances and includes few sources that can be suspected to distort a true depiction of the physiological events to be measured At the same time, the method offers high versatility to meet the many different problems appearing in clinical use

Psycho-acoustic differential tests With respect to the traditional test used, speech discrimination test, Fowler's recruitment test and tone threshold decay test, the results taken as a whole show a poor reliability for the identification of retrocochlear disorders This drawback is earlier known but nevertheless, the low test scores obtained in this study seem surprising in view of the very modest requirements for a test result to be classified as pathologic A thorough review of this circumstance is found in a complementary study (Hirsch & Anderson, 1980)

Pathologic reflex threshold elevation When encountering a pathologically elevated reflex threshold, one must be aware that such a condition can have its origin in a variety of causes Although here we are only concerned with reflex threshold elevation caused by disturbances of the afferent branch of reflex arc, it must always be kept in mind that such an elevation also can arise from the dysfunction of the motoric stapedius arc in the recording (opposite) ear or from an unnoticed situation in the stimulus ear in the form of a minor conductive dysfunction (Anderson & Barr, 1967) or even from a trivial situaiton such as the occlusion of the ear canal by the pressure of the earphone (Chandler, 1964) Such irrelevant sources of reflex threshold elevation are to be avoided and are possible to control by a carefully planned technique of examination

As pointed out in the introduction many studies, such as the present, are in agreement with respect to the close connection existing between the elevation of stapedius reflex threshold and the presence of retrocochlear disorder One specific group of cases, however, in this respect constitutes an intricate problem, namely cases with hearing dysfunction of genetic or hereditary origin It has been shown that pathologic reflex threshold elevation is far from uncommon in such cases and the condition has also been described as characteristic in normal hearing carriers of genes for deafness (Andersson & Wedenberg, 1968) The organic basis for this has found no explanation but it is known that these hereditary afflicted cases, although recording pathologically elevated reflex thresholds, demonstrate no other audiometric finding or symptoms usually associated with retrocochlear lesions Thus, it still remains an open question whether the pathology in the hereditary cases is localized to the retrocochlear structures. It is

SUMMARY

A study of stapedius reflex threshold elevation and reflex decay has been performed in patients with moderate hearing loss (≤ 60 dB) referred for examination on the suspicion of a possible retrocochlear process

In 564 cases studied, 110 were found to have pathologically elevated reflex thresholds (= 19 5%, mean HL 30 8 dB) In 40 of these 110 cases the existence of a retrocochlear lesion was verified In the remaining 70 cases where the hearing dysfunction could not be tied to a specific aetiology, no less than 25 cases (=36%) demonstrated elevated stapedius reflex thresholds bilaterally

With the study material extended to 1366 patients, the occurrence of pathological reflex decay (RD+++) was investigated RD+++ was found in 41 cases (= 3%, mean HL 29 1 dB) In more than half of these cases the presence of a retrocochlear lesion was confirmed

In the 41 cases selected on the basis of RD+++, the proportion of positive results in the psycho-acoustic tests indicative of a retrocochlear lesion was similar in the groups with an unspecific aetiology and those with a verified retrocochlear pathology This is in contrast to the findings in the 110 cases with pathologic reflex threshold elevation where the aetiologically unspecific groups showed a low proportion of positive outcomes in the psycho-acoustic tests

When analyzing the two reflex parameters separately it was found that 1 case out of 5 with pathologic stapedius reflex threshold elevation and 1 case out of 3 with RD+++ was a cerebello-pontine angle tumour case

The probability of encountering normal stapedius reflex characteristics in a case of CPA tumour seems very slight All of the 34 CPA cases of the present study showed stapedius reflex abnormalities either in the form of reflex threshold elevation (including reflex threshold level beyond limit of audiometers'maximum output) or RD+++ or a combination of reflex threshold elevation and RD+++ In the 18 CPA cases where the reflex decay test was applicable, 14 cases demonstrated RD+++ Among the 4 remaining cases, 3 showed a degree of reflex decay hitherto not regarded as pathologic

For obtaining reliable results in stapedius reflex testing, the technique used is of vital importance and recording artefacts reported for some types of equipment must be avoided It is exemplified that the use of a too high SPL of the carrier tone (probe tone) can distort the true reflex decay pattern, thus rendering the result of the reflex decay test misleading In the light of these facts, the technique and procedure applied for stapedius reflex measurement in this study is discussed

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde an Patienten die wegen Verdachts eines eventuellen retrocochlearen Prozesses uberwiesen worden waren der Stapediusreflex studiert und zwar insbesondere mit Hinblick auf Erhohung der Reflexschwelle und zeitlichen Abfall des Reflexes (reflex decay) Die Patienten hatten durchgehend im Verhaltnis massige Horverluste (≤ 60 dB HL)

Von den Patienten wurden 564 Falle auf pathologische Erhöhung der Reflexschwelle untersucht und in 110 der Falle (d h in 19 5% mit mittlerem HL von 30 8 dB) eine solche konstatiert In 40 von diesen 110 Fallen konnte das Vorliegen eines retrocochlearen Schades verifiziert werden Von den restlichen 70 Fallen bei denen keine spezifische Atiologie für die Gehorsdysfunktion gefunden werden konnte war die Erhohung der Reflexschwelle in 25 Fallen (d h in 36%) bilateral

Die obigen 564 Falle gingen als Teilmaterial in ein Gesamtmaterial von 1366 Patienten ein die in den letzten 10 Jahren auf das Vorliegen von pathologischem Reflexabfall untersucht worden sind In 41 Fallen (d h in 3% der Falle mit mittlerem HL von 29 1 dB) wurde ein ausgepragter Reflexabfall (als RD+++ bezeichnet) festgestellt in mehr als der Halfte dieser Falle wurde ein retrocochlearer Schaden sichergestellt Die beiden Reflexteste separat betrachtet hatten damit zum Ergebnis von den Fallen mit pathologischer Reflexschwellenerhohung hatte einer von 5 Patienten und von denen mit RD+++ – Indikation einer von 3 Patienten einen Tumor der hinteren Schadelgrube

In den psycho akustischen Testen war bei den 41 Fallen mit pathologischem Reflexabfall der relative Anteil an positiven Testresultaten d h solchen die auf einen retrocochlearen Schaden deuten ungefahr der gleiche in den Fällen bei denen keine spezifische Atiologie vorlag und in den Fällen mit verifizierter retrocochlearer Pathologie Im Gegensatz dazu zeigten die psycho-akustischen Teste in der Gruppe mit pathologische erhöhter Reflexschwelle (110 Falle) in den Fallen mit nicht spezifischer Atiologie nur einen geringen Anteil positiver Testresultate

Aus der vorliegenden Untersuchung ist zu entnehmen dass die Wahrscheinlichkeit durchgehend normaler Stapediusreflexcharakteristika in Fällen von Tumoren die den N acusticus affiziert haben sehr gering ist Alle 34 solche Tumorenfalle hatten anormale Reflex Responsen und zwar entweder in Form einer Reflexschwellenerhohung (inbegriffen die Fälle bei denen die Reflexschwelle uber den aussersten Audiometerniveaubereich 120 dB HL hinaus erhöht war) oder als RD+++ Indikation oder aber sie zeigten sowohl Reflexschwellenerhohung wie RD+++ Von den 18 Tumorenfällen in denen der Reflexabfall gemessen werde konnte hatten 14 Fälle einen RD+++ Abfall Von den übrigen 4 Fallen hatten 3 einen als RD++ bezeichneten Abfall der jedoch nicht direkt als pathologisch indikativ angesehen werden kann

Abschliessend wird besonders darauf hingewiesen dass bei den Stapediusreflextesten die apparative Messanordnung und die messtechnische Durchführung von ausschlaggebender Bedeutung zur Sicherstellung zuverlässiger Resultate ist So wird gezeigt dass ein zu starker [illegible] (probe tone) zu Verzerrungen des eigentlichen Verlaufs des Reflexabfalles und damit zu falschen Schlüssen führen kann

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Audiologic Test Results in 96 Patients with Tumours Affecting the Eighth Nerve

A clinical study with emphasis on the early audiological diagnosis

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INTRODUCTION

Patients with tumours localized to the cerebello-pontine angle constitute about 11% of all cases of intracranial tumours and the majority of these tumours, about 75%, originate from the eighth nerve (Lundborg, 1952, Nager, 1969, Pool et al, 1970) With respect to terminology, these tumours appear in the literature under a great number of different designations, either in more general terms of localization such as cerebello-pontine angle tumours, eighth nerve tumours or acoustic (nerve) tumours or under denominations aiming at characterizing the morphological changes, such as acoustic neurinomas (or neuromas), schwannomas, neurofibromas, angioneurofibromas, perineural gliomas, amongst others (Gruskin & Carberry, 1979)

Further, the compass of such expressions as eighth nerve tumour and acoustic tumour is not altogether distinct as some authors use these terms not only for tumours emerging from the eighth nerve structures but also for other tumours affecting the function of this nerve With few exceptions, the eighth nerve tumours are of unilateral occurrence but in some 5% of the cases they are found bilaterally, usually in patients suffering from von Recklinghausen's disease (Nager, 1969, Morrison, 1975, Gruskin & Carberry, 1979) Among other tumours originating in the cerebello-pontine angle region, meningeomas, cholesteatomas and gliomas are those types most frequently enocuntered (Nager, 1969)

An estimation based on the records of the Swedish Cancer Registry shows that approximately 55 cases of diagnosed acoustic neurinomas can be expected to occur annually in Sweden, which calculated on a population of just over 8,000,000 shows an incidence of about 0 7 per 100,000 Approximately the same incidence was reported for the population of Great Britain by Morrison (1975)

The reported morbidity stands in sharp contrast to the incidence of acoustic neurinomas found in studies of post mortem materials where a frequency of 1 per 100 cases or even more has been reported (Hardy & Crowe, 1936, Moberg et al, 1969) This extreme discrepancy has not been satisfactorily accounted for but it has been suggested that an acoustic neurinoma can cause vascular or biochemical disturbances in the end organ resulting in degenerative changes in the membranous labyrinth, thereby causing hearing impairment with predominantly cochlear symptoms (Dix & Hallpike 1950, De Moura, 1967, Schuknecht, 1974) Another proposed explanation is that some acoustic neurinomas do not invade the cochlear fibres of the nerve and not causing the characteristic hearing symptoms thus remain undiagnosed (Ylikoski et al, 1979)

Quite logically the primary complaints in patients with acoustic neurinomas are symptoms from the cochlear branch of the eighth nerve in the form of tinnitus or progressive sensorineural hearing impairment, and from the vestibular branch appearing as balance disturbances As the process advances, the tumour can affect the adjacent facial and trigeminal nerves, giving rise to sensory or motoric symptoms from the distribution areas of these nerves Eventually, signs of increased intracranial pressure can arise as well as symptoms, even bilateral indicating involvement of brainstem and cerebellum

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advanced tumour cases has been well established (Dix & Hallpike, 1960, Aschan & Hugosson, 1967) However, in cases of small tumours opinion on the diagnostic capacity of vestibular tests varies considerably According to Lundborg (1952) 68% of ears with small acoustic tumours showed normal caloric reactions These examinations were, however, not performed with ENG recording Fisch & Wegmüller (1974) in their paper on the 'early' diagnosis of such tumours, report significantly reduced caloric ENG responses in not less than 94% of 33 patients examined King et al (1976), using caloric stimulation and ENG recording, report that '20% of the patients with tumours under 2 cm in diameter have an equal duration of nystagmus with each ear'

In contrast to these promising results stands the report by Sheehy and Inzer (1976) who found only 6 of the 13 ears with small acoustic tumours in their series to have clearly pathologic ENG responses This proportion agrees with the findings by Linthicum et al (1979) who report that only 43% of the small acoustic tumours in their series demonstrated reduced vestibular responses

Thus, the reported results are too inconsistent to permit an evaluation of the diagnostic capacity of caloric vestibular tests in cases of small acoustic tumours Further, the technique commonly applied in clinical practice seems not particularly suited to distinguish between reduced caloric responses caused by end organ dysfunction and those caused by nerve trunk disorder

For the diagnosis of disorders in the cochlear branch of the eighth nerve, a great number of differential diagnostic hearing tests has been devised, some of them have survived and are included in the conventional psycho-acoustic audiometric test battery of most clinics Although these tests in general have been considered as fairly accurate to differentiate cochlear from retrocochlear disorders, exceptions from this rule are not few and some authors even regard the reliability of such tests totally insufficient in the diagnosis of acoustic neurinomas (Shapiro & Naunton, 1967)

Table I surveys the results of some common psycho-acoustic tests in some representative series of verified cases of tumours affecting the eighth nerve Included in the table are also results from one of the objective tests available The stapedius reflex test For reasons that will be more closely considered in the discussion, this compilation can only give a rough outline of the situation However, a cursory glance at the results reported for the psycho-acoustic tests reveals that the proportion positive scores averages only some 50%, i e the tests have failed in about every second tumour case Considering the fact that these low scores were obtained in materials including advanced tumour cases, it seems justified to raise the question whether the sensitivity of the conventional psycho-acoustic tests is not totally inadequate for detection of tumour cases at an early stage, while the hearing is still normal or the hearing loss insignificant

In addition to the battery of psycho-acoustic diagnostic tests, most clinics today also utilize some objective test methods for the diagnosis of eighth nerve tumours The stapedius reflex test and Electric Response Audiometry (ECochG, BERA) Promising results have been reported with the latter techniques (Rosenhamer 1977, Gibson, 1978 Thomsen et al , 1978, Clemis & McGee, 1979, Selters

In large surveys, a general relation between size of tumour and severity of symptoms can be demonstrated (Lundborg, 1952, Johnson, 1979) but the exceptions from this general tendency are numerous Patients with large tumours may display only few and mild symptoms whereas an intracanalicular tumour of minimal size can cause severe deafness and extinguished vestibular function

As the primary symptoms usually arise from the eighth nerve, the patient in most cases turns to the otologist or a general practitioner for advice To quote Herbert Olivecrona (1969), the famous neurosurgeon 'Education of the doctors, general practioners and otologists, who are likely to be the first to see patients harbouring acoustic tumours, will probably be the most important factor in improving the result of surgery' Thus, it is the delicate task of the otologist or general practitioner to identify, among the large number of patients seeking advice for diffuse hearing or balance symptoms, the possible tumour cases Here, a complicating circumstance is that the rare cases of eighth nerve tumours mainly are found in the age groups where hearing and balance problems of various origin are far from uncommon Still, by maintaining a sharp alertness to the symptoms some clinicians succeed in picking out an impressive number of such tumour cases (Axelsson et al , 1971)

For patients with symptoms of a tumour affecting the eighth nerve, the definite diagnosis rests on neuro-radiological examinations For larger acoustic neurinomas, conventional radiography (with or without tomography) has been reported to identify pathological changes in the internal auditory meatus in 78 to 95% of the cases (Crabtree & Gardner, 1979) Examination with computed tomography (CT scanning) utilizing contrast enhancement, has been stated to give identification scores as high as 90% for tumours measuring more than 1 5 to 2 0 cm in diameter For tumours of less than this size, the method is not regarded as sufficiently reliable (Morrison, 1975, Parker & Davis, 1977, Witten & Wade, 1979) although reports on a modified CT-scanning technique raise expectations of considerably improved resolution in the future (Hanafee et al , 1979) For the visualization of smaller tumours as well as determination of actual size, shape and position, encephalography is applied, and for highest possible resolution, cisternography with positive contrast enhancement (Grepe 1974, Morrison, 1975)

As it must be regarded impractable to submit every patient presenting a possible eighth nerve problem to extensive neuro-radiological examinations, efforts have been made to design tests that reveal such functional disturbances in the eighth nerve that can be expected to arise from a tumour An early diagnosis has become increasingly important as the acoustic neurinomas, while still small, can be removed by new lenient surgical procedures (House, 1961, Rand & Kurze, 1967) or even successfully treated by non invasive stereotactic radiosurgery (Hirsch et al , 1979) Consequently interest has been focused on diagnostic tests sensitive enough to reveal disturbances in the vestibular and auditory function at the earliest possible stage

Examination of the vestibular function by caloric stimulation was early described as a diagnostic test in cases of cerebello pontine angle tumours (Cushing, 1917) That such examinations are of indisputable value in the diagnosis of

advanced tumour cases has been well established (Dix & Hallpike, 1960, Aschan & Hugosson, 1967) However, in cases of small tumours opinion on the diagnostic capacity of vestibular tests varies considerably According to Lundborg (1952) 68% of ears with small acoustic tumours showed normal caloric reactions These examinations were, however, not performed with ENG recording Fisch & Wegmüller (1974) in their paper on the 'early' diagnosis of such tumours, report significantly reduced caloric ENG responses in not less than 94% of 33 patients examined King et al (1976), using caloric stimulation and ENG recording, report that '20% of the patients with tumours under 2 cm in diameter have an equal duration of nystagmus with each ear'

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Table 1 *Survey of hearing test results in representative series of cases with tumours affecting the eighth nerve Percentage figures refer to proportion of cases tested in which the test results was reported as positive (i e indicating presence of a retrocochlear lesion), actual number of cases tested in brackets Positions denoted – indicate that information is missing or that the presentation of stated results does not permit interpretation Direct comparisons between studies are seldom possible, see Discussion*

Author	Year	Cases	Mean HL	PSYCHO ACOUSTIC TESTS					STAPEDIUS REFLEX TESTS		
				Speech discr	Fowler (ABLB)	Tone decay	Bekésy III & IV	SISI	Thresh	Decay	Thresh and/or decay
Johnson	–77	500	(75 totally deaf)	–	50% (171)	40% (304)	57% (363)	55% (362)	52% (75)	69% (36)	85% (75)
Clemis	–76	121	> 50 dB in 64 cases	–	57% (71)	77% (79)	47% (83)	60% (64)			–
King et al	–76	60?	–	78% (–)	< 90% ( )	80% ( )	76% (–)		94% (60)	100% (18)	94% (60)
Fisch & Wegmuller	74	33	> 50 dB in 27 cases	55% (23)	–	–	30% (17)	–	40% (–)	40% ( )	
Thomsen & Terkildsen	–75	32	< 80 dB		77% (30)	73% (15)	70% (10)	–	87% (30)	60% (5)	87% (30)
Jerger et al	–74	30	< 80 dB				47% (30)		63% (30)	36% (11)	77% (30)
Sheehy & Inzer	–76	24	24 dB	0% (24)		–			46% (24)	62% (13)	80% (24)
Palva et al	–78	23	69 dB?	–	63% (–)	66% ( )	54% ( )		71% ( )	–	71% (–)
Liden & Korsan Bengtsen	–73	22	–	64% (–)		69% (13)	50%? (–)	–	100% (22)		100% (22)
Anderson et al	–70	21	< 60 dB	38% (21)	62% (18)	45% (20)			95% (21)	92% (13)	95% (21)

& Brackman, 1979) but as these test methods only fairly recently have been introduced in clinical routine, no case material of sufficient size is yet available to judge the over-all reliability and specificity with respect to eighth nerve tumour cases

Regarding stapedius reflex measurements which now have been used as clinical diagnostic tests for a great number of years, enough experience has been gathered to permit an evaluation of such measurements in the early diagnosis of tumours affecting the eighth nerve Metz (1952) was the first to report the pioneering observation, made in two cases of acoustic tumours that acoustic stimulation of the affected ear did not result in recordable stapedius muscle contractions This absence of responses was in contrast to the results he obtained in ears with other types of sensorineural hearing loss where he observed that stapedius reflexes could be elicited in spite of the hearing impairment Metz assumed a connection to exist

retrocochlear origin by Thomsen (1955)

With further development of the technique and procedures for stapedius reflex measurement, the method became available for most clinics and with the years a number of studies dealing with the behaviour of the stapedius reflex threshold in cases with tumours affecting the eighth nerve has been published (Table I) With few exceptions, these papers support the conception of the stapedius reflex threshold test as a more sensitive indicator for the identification of eighth nerve tumour cases than the psycho-acoustic differential diagnostic hearing tests

Another parameter of the stapedius reflex response that has been submitted to study with respect to its diagnostic capacity is the persistence of reflex response

they had observed an abnormal decline in reflex response amplitude for tones presented at a stimulus level 10 dB above the individual reflex threshold level and with a stimulus duration of 10 s The authors designated the phenomenon as 'reflex decay' and furnished evidence that the defect causing the reflex decay phenomenon was to be found in the afferent part of the stapedius reflex arc and they also formulated a criterion for the rate of amplitude decline to be classified as pathologic

These original findings on the clinical application of the reflex decay phenomenon as a test for retrocochlear disorders have been followed by a number of studies by other authors (*cf* Table I) and although some criticism has appeared (Chiveralls, 1977) the reflex decay test in general seems to have been accepted as a valuable tool in the diagnosis of eighth nerve tumours

Results from stapedius reflex threshold tests and reflex decay tests in a series of tumours affecting the eighth nerve are compiled in Table I The same reservations as mentioned above apply with respect to the interpretation of stated test results. These matters will be returned to in Discussion

Based on their experiences in the original investigation, Anderson et al (1969) put forward two proposals of especial interest for the present study, namely (i) that the stapedius reflex decay possibly can be the earliest audiometric indication of an incipient retrocochlear lesion and (ii) that pathologic reflex decay seemed to have a surprisingly high specific connection to retrocochlear lesions caused by tumours These suggestions were to some extent supported by a recent study (Hirsch & Anderson, 1980)

The object of the present paper is to study, in an extended material of cases with tumours affecting the eighth nerve, the reliability, sensitivity and specificity of the stapedius reflex tests and the reflex decay tests with special regard to the early diagnosis of such processes, further, to relate the findings from the two reflex tests to the results from some commonly used psycho acoustic differential diagnostic tests The speech discrimination test, the Fowler recruitment test (ABLB test) and the tone threshold decay test

TECHNIQUE

Recently, a thorough description of the equipment and procedures applied in the laboratory examinations has been presented (Hirsch & Anderson, 1980) The reader is referred to this paper for further details, in particular with respect to the methods for tympanometry, stapedius reflex examinations and tone threshold decay recording Below are only summarized some of the more distinguishing features of the methods used

Equipment

Test rooms All hearing tests and reflex measurements were performed with the patient seated in a satisfactorily sound insulated chamber with test equipment and operator in an adjoining room

Audiometers As the study covers quite a number of years, many different audiometers have been used for the AC/BC-audiometry, for stapedius reflex elicitation, and for the various differential hearing tests Irrespective of type of audiometer, it has been carefully checked that the equipment fulfilled the demands set up in IEC Standard 177 (1965) and that the calibration conformed to ISO Recommendation R 389 (1964) When masking was applied, the noise-characteristics and procedures described by Lidén et al (1959) were used

Tympanometry and stapedius reflex recording were performed with the technique originally described by Anderson et al (1956) and with the method developed to its present state by Klockhoff (1961), Anderson (1969) and Hirsch & Anderson (1980) The methods operate with a carrier tone frequency of 500 to 800 Hz (nominal 550 Hz) at an intensity corresponding to 60 dB HL Strictly speaking, the technique used cannot be regarded as an actual acoustic impedance measurement (which preferably involves separation of reactive and resistive components) but rather as a recording of the amount of reflextion of the carrier tone

All changes in the amplitude of sound reflexion of the carrier tone, as a result of tympanometric pressures or reflex activity, are logarithmically recorded as dB deviation from the ear's steady state reflexion The positive and negative air pressure used for tympanometry is applied as brief pulses with a build up time of 3 s, starting from ambient air pressure up to a positive or negative standard pressure of 10 mm Hg (= 136 mm H_2O) ('transient pressure course', Anderson, 1977, Tyler, 1979) The air pressure device can be set to maintain automatically, and with high accuracy, any positive or negative pressure over infinite period of time, a procedure used for manoeuvering the eardrum into a suitable state for reflex recording

Methods criteria

[illegible]

[illegible] the hearing could be regarded as normal for age *Tone thresholds* better than the median values for corresponding sex and age group in WSFHS (Glorig et al , 1957) were accepted as normal

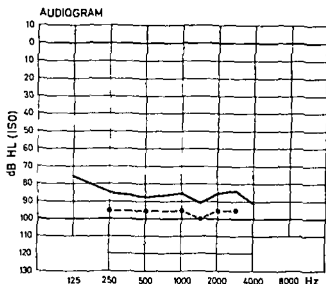


Fig 1 Normal stapedius reflex threshold (continuous) and curve indicating first pathologic value (dashed) To be classified as pathologically elevated the reflex threshold should reach or exceed the limit curve on at least 4 of the 6 test frequencies (Anderson & Wedenberg 1968)

Speech discrimination test Phonetically balanced Swedish monosyllabic words according to Lidén (1954) were used as test material Test results were evaluated empirically against size and shape of hearing loss Fifty words were presented at each level tested and results are given as maximum per cent correctly repeated words

Fowler s test Pair of tones (duration 1 s interval 0 25 s) were presented and the patient was instructed to judge loudness after each pair The HL of the pulses to the control ear was varied until loudness balance was obtained The procedure was repeated on at least two levels for each frequency tested The hearing impairment was judged to be non recruiting if an imbalance of 15 dB or more (calculated as the mean of the imbalance at 0 5 1 0 and 2 0 kHz) could be demonstrated in the HL range 80–120 dB

Tone decay was graphically recorded according to the method designed by Anderson (1960) and described by Hirsch & Anderson (1980) The outcome was judged as pathologic if the tone threshold shift over 90 s exceeded any of the following values 25 dB at 2 kHz 15 dB at 1 kHz and 10 dB at 0 5 kHz

Tympanometry performed with transient pressure technique was utilized to ensure that the eardrums bilaterally were in suitable condition for the stapedius reflex test

Stapedius reflex measurements were carried out by contralateral stimulation with the standard octave and half octave test frequencies 0 25–4 kHz As stape-

dius reflex thresholds were recorded the lowest HL where repeatable responses were seen in the recording As pathologic limit for the reflex threshold was used the criterion set by Anderson & Wedenberg, 1968 (Fig 1) Absence of stapedius responses from the recording ear was not accepted as caused by a disturbance of the afferent arc of the (opposite) stimulus ear, unless stapedius activity could be demonstrated by non acoustic stimulation (Klockhoff & Anderson, 1959)

Reflex decay was determined at a stimulus level of 10 dB above the individual reflex threshold by presenting the tone stimulus for 10 s at 0 5, 1 0 and 2 0 kHz with 10 s interval between stimuli Pathologic reflex decay (RD+++) was judged to be present if the response amplitude (logarithmically recorded) was found to decline more than 50% in 5 s on 0 5 and 1 0 kHz If more than 50% reflex decay was recorded for 1 kHz stimulation but not for 0 5 kHz, the reflex decay was classified as RD++ If decay was clearly visible in the recording but did not meet the 50% criterion for 0 5 or 1 0 kHz, the reflex decay was classified as RD+ In this study, RD++ and RD+ were not regarded to fulfil the criterion of pathology (Hirsch & Anderson, 1980) On suspicion of motoric origin for the reflex decay this was checked by tactile elicitation or two-tone test (Gjaevenes & Sohoel 1966)

The above described test procedures as well as criteria for evaluation of test results have been adopted from the studies by Anderson et al (1970) and Hirsch & Anderson (1980), hence results presented in the present paper are directly comparable to these two studies

MATERIAL

This study concerns cases of tumours affecting the eighth nerve presenting a maximum hearing loss of 60 dB on the affected side (calculated as the mean of the tone threshold at 0 5, 1 0 and 2 0 kHz) and where middle ear conditions permitted application of stapedius reflex examinations The limit of 60 dB was set because the aim was to study the capacity of the various hearing tests in the early diagnosis of such processes, if possible even before an actual hearing loss had occurred In limited parts this case material has been the subject of earlier studies (Anderson et al , 1970, Hirsch & Anderson 1980)

The entire material consists of 96 patients with 97 tumours 75 tumours were classified as acoustic neurinomas and 22 as other cerebello-pontine angle tumours *The total group of tumours in the following will be referred to as* CPA tumours, with the sub-grouping AN for the acoustic neurinomas and CPA O for the other tumours in the cerebello pontine angle This latter group is confined to cases where the CPA O tumour, although not emerging from the eighth nerve, still had given recordable symptoms from this or its ascending pathways

In the AN group five (possibly six) of the patients suffered from von Recklinghausen's disease In three of these cases tumours were diagnosed bilaterally However, two of these altogether six affected ears showed a hearing loss exceeding the limit of 60 dB, leaving only one case with bilaterally studied ears in the case material

At the time of diagnosis, 28 cases displayed hearing thresholds within normal limits with respect *to age and sex 14 of these patients did, however, report* subjectively impaired hearing function In the 82 patients with subjective or objective signs of hearing impairment, information concerning onset was obtained in all but *one The duration of the hearing* complaint ranged from a few weeks up to 24 years, the average duration being in the order of 2 years (Table II) In 7 patients the hearing impairments were of sudden onset, these were all AN cases In 63 cases hearing impairment or tinnitus were the first symptoms Distribution of *primary and secondary symptoms is given in* Table III

Table II *Duration of hearing dysfunction (N = 96 ears)*

Duration	No of ears
6 months or less	26
7– 12 months	19
1– 3 years	20
4– 6 years	8
7– 9 years	5
10–12 years	3
>12 years	1
No hearing dysfunction	14

Table III *The presenting symptoms (N = 96 ears)*

Symptom	Primary	Secondary
Deafness/tinnitus	63	19
Unsteadiness	18	28
Vth nerve symptoms	7	12
Earache	2	14
Other	6	–

With respect to the patients' age, this ranged from 5 to 81 years with a mean age of 43 years (Fig 2) Fortytwo of the tumours were rightsided and 55 leftsided, 44 of the patients were women, 52 men

In the 60 tumours removed by open surgery, the classifications were verified by histological examination In the 25 tumours treated by stereotactic radiosurgery and the 12 non operated tumours, classifications were based on the results of the neuro-radiological examinations The 22 cases of the CPA-O tumour group were classified as follows 6 meningeomas, 6 gliomas or astrocytomas, 4 cholesteatomas, 2 neurinomas (emerging from cranial nerves other than the eighth), 2 metastatic tumours, 1 angioma and 1 tumour of unknown histological type With respect to tumour size, 38 of the ANs measured 2 cm in diameter or less whereas the CPA-O tumours in general were reported to be of greater size

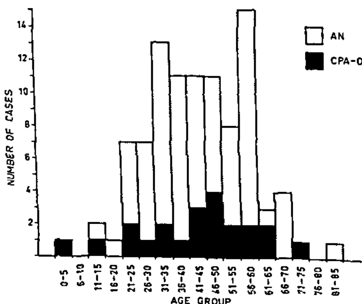


Fig 2 Age distribution of the case material AN = acoustic neurinomas CPA-O = other cerebello-pontine angle tumours affecting the eighth nerve

With the exception of 4 cases, where the sensorineural impairment was superimposed by a conductive disorder and consequently stapedius reflex measurements were out of the question, the group of AN cases reported here constitute all such cases which during the years 1961 to 1979 were referred to the Department of Audiology, Karolinska Hospital, for diagnosis and who fulfilled the above criterion with respect to hearing function

RESULTS

Audiological examination

The average tone threshold hearing loss (given as mean at the test frequencies 0 5, 1 0 and 2 0 kHz) in the total material of 97 ears affected by CPA tumours was 32 1 dB Twentyeight of the patients had hearing thresholds within normal limits with respect to age and sex on the affected side (according to the criteria presented on page 7) The shape of the hearing threshold curve showed in 39 cases a high tone loss in 5 cases a low tone loss in 6 cases a flat loss in 10 cases the curves were peak shaped and in 9 cases trough shaped

The results of the differential diagnostic audiological tests used are seen in Table IV The low sensitivity in the psycho-acoustic tests is clearly demonstrated, a findig that is in agreement with earlier studies

Table IV *Differential hearing tests (N = 97 ears) Results indicating retrocochlear lesion in per cent of ears tested*

Speech discr test	Tone decay test	Fowler's test	Stap refl test
45%	53%	64%	98%

As stated earlier, the following two characteristics in the reflex status were used in the classification of the results abnormal elevation of the reflex threshold and pathologic reflex decay (RD+++) The distribution of the different characteristics recorded in the total material are given in Table V, where it is seen that in the majority of ears (60%) the reflex threshold could not be reached even at the maximum stimulus intensity, consequently, with respect to the outcome of reflex test, this total absence of responses seems to be the most pronounced characteristic in the ears affected by eighth nerve tumours About one-third of the cases showed a combination of ele [illegible]
3 cases reflex decay was rec[illegible]
these cases had normal hear[illegible] [illegible] cases showed completely normal reflex results and are discussed in Case reports, page 15

Table V *Outcome of the stapedius reflex tests (N = 97 ears)*

Reflex threshold		Reflex decay +++	++	+	–
Above limit of audiometer	58	(Not applicable)			
Elevated	34*	20	6	2	3
Normal	5	3	1	0	1

* Reflex decay test not applicable in 3 cases due to elevation of reflex threshold

The reliability of audiological differential tests in relation to hearing loss To investigate whether the poor sensitivity in the psycho-acoustic tests (Table IV) was biased by the use of a selected material of cases with average fairly good hearing thresholds, the total material was subdivided in two groups 36 cases with hearing loss ≤ 32 dB and 39 cases with hearing loss 33 to 60 dB Table VI shows a clear tendency towards higher reliability for the psycho-acoustic tests with increasing hearing loss With respect to the results of reflex measurements, both groups score so near maximum that no relevant differentation is possible However, when considering the details of reflex response characteristics in the two groups, it is clear that the proportion of ears with reflex thresholds above the audiometer's limit is considerably higher in the group with hearing loss 33 to 60 dB (69%) than in the hearing-loss group ≤ 32 dB (47%)

Table VI *Differential hearing tests in relation to mean tone-threshold loss Results indicating retrocochlear lesion in per cent of ears tested Case material of acoustic neurinomas divided in two groups*
Group A, HL ≤ 32 dB, Group B HL = 33–60 dB

	Speech discr test	*Tone* decay test	*Fowler's* test	*Stap* refl tests
Group A (N = 36) (M HL = 19 4 dB)	37%	50%	55%	94%
Group B (N = 39) (M HL = 47 5 dB)	55%	62%	73%	100%

Audiological test results in AN versus CPA O cases To investigate whether the audiological test pattern in some specific way differed between the AN and the CPA-O tumour cases, the test results were analyzed with respect to aetiology With regard to tone threshold pattern, the only significant difference found was a higher proportion of normal hearing thresholds in the CPA-O group (50%) about twice that of the AN-group (23%) In addition to that, all other threshold patterns are represented in both groups Nor with respect to the results of the psycho-acoustic tests are any remarkable differences of interest noted between the two groups (Table VII) The slight tendency towards higher scores in the psycho-acoustic tests for the AN-group can very well be explained by the somewhat more pronounced hearing threshold loss in this latter group (*cf.* Table VI) Further, Table VII indicates a slightly higher score in the stapedius reflex measurements in the CPA-O group in spite of the lower hearing threshold level in this group A detailed analysis of the results here reveals a considerably higher proportion of non-elicitable reflexes in the CPA O group (14 out of 22 ears) than in the AN-group (44 out of 75 ears) Also, the proportion of ears with normal hearing thresholds in combination with pathologic reflex thresholds was markedly higher in the CPA-O group

Table VII *Differential hearing tests in case of acoustic neurinomas (AN) and other cerebello pontine angel tumours (CPA O) Results indicating retrocochlear lesion in per cent of ears tested*

	Speech discr test	Tone decay test	Fowler's test	Stap refl tests
AN cases (N = 75) (M HL = 34 0 dB)	47%	56%	63%	97%
CPA O cases (N = 22) (M HL = 25 5 dB)	35%	40%	67%	100%

Results of some non audiological tests

During the long period of time that the material of the present study has been collected, the audiological test procedures and criteria have been held constant, however, this is not the case with other non audiological examinations Other test routines and new methods have been introduced, and this development has been most pronounced in the field of radiology However, two test procedures have been reasonably consistent over the years with respect to technique and systema tically enough carried out to permit a summary of the results These are, testing of the vestibular caloric reactions by ENG and conventional radiography and tomography of the internal auditory meatus

The caloric reactions were recorded by ENG and evaluated by an ENG-specialist according to the criteria current at the time the examination was made A survey of the medical files revealed that in the AN group 70 cases had been submitted to caloric tests 35 of the affected ears showed no caloric response 30 ears reduced responses and 5 ears had been judged as normal in this respect Among the 11 tested cases in the CPA O group 4 of the affected ears showed no responses 6 ears reduced responses and 1 ear normal responses

Conventional radiography and tomography of the internal auditory meatus is mainly of interest in the AN group In 66 of the 75 ears affected by AN, tomo graphy was performed and in 45 cases (68%) the neuro-radiologic specialist re-ported pathological changes of the internal meatus

Case reports

In only two cases the stapedius reflex tests failed to identify the tumour In both cases the reflex thresholds were accepted as normal and although some reflex decay was recorded in case B the decay was too small to fulfil the criterion for pathology A compilation of the results is shown in Fig 3 and in Table VIII

Case A left ear This case was a man with von Recklinghausen's disease Sur gery had previously been performed because of fibromata in the cervical and thoracic spine At the time of our first examination he was 30 years old and had a rightsided deafness for 6 years and a history of hearing dysfunction in is left ear for 10 years Audiological examination showed total deafness in the right ear and a high tone loss in the left ear Neuro-radiological investigation identified tumours

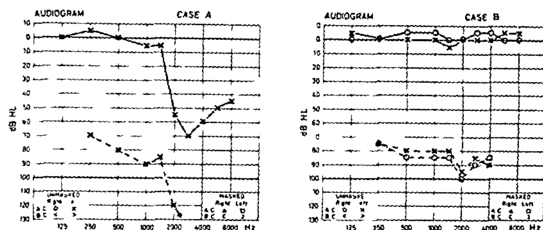


Fig 3 Audiograms in the two cases of acoustic neurinomas where the reflex tests did not indicate a retrocochlear dysfunction Case A suffered an earlier total deafness on the opposite right ear Case B had a left sided tumour Reflex thresholds = dashdotted curves

Table VIII *Compilation of test results in the two cases of acoustic neurinomas where the audiologic examination failed to identify retrocochlear lesions*

	Case A	Case B
Speech discr	100%	98%
Tone decay	< 5 dB/2kHz	5 dB/2kHz
Fowler's test	– *	Normal
Reflex decay	0	++
Cal response	Reduced	Reduced
X ray porus	Enlarged	Enlarged
CFS-protein	0 73 g/l	0 99 g/l
Tumour size	~ 20 mm	~ 30 mm

* Opposite ear deaf

at both sides the right one about 13 mm the left about 20 mm in size The left-sided tumour was treated by stereotactic radiosurgery

Case B right ear A 23 year old woman with 3 attacks of vertigo lasting for several days in the last half year She had no complaints of hearing dysfunction Pneumoencephalography revealed a tumour of about 30 mm adjacent to the right internal meatus Surgery was performed and an acoustic neurinoma extirpated

It is interesting to note that in these two cases – both belonging to the younger category – none of the audiologic tests fulfilled the pathologic criteria set forth, however although not formally fulfilling the criterion the presence of a RD++ in case B must of course be regarded as an alarming signal

DISCUSSION

As touched upon in the Introduction, the need for an early diagnosis of eighth nerve tumours has become increasingly important Following the development of new radiosurgical methods, suitable for the treatment of such tumours while still of small size (Hirsch et al 1979) the trend towards an early diagnosis has been particularly pronounced in this hospital

In collecting the case material of the present study, a criterion was set with respect to hearing, namely, a hearing loss in the affected ear not exceeding 60 dB as mean for the three middle test tone frequencies. This criterion was chosen with the intention of obtaining a selected material suitable for the study of the capacity of audiological tests at the early stages of these tumours. That this endeavour has been met with success is proven by the fact that nearly one third of the cases presented hearing thresholds within normal limits half of the case material had mean threshold values that had not reached what usually is regarded as border of practical hearing handicap

The hearing threshold curve configuration is of little value in identifying the early CPA tumour Although the high tone loss constitutes the largest group it still contains less than one-third of the cases. Among the remainder, a variety of different threshold pattern emerged Nor does the mode of onset provide reliable information for the diagnosis. It is true that in most cases the onset is of the expected insidious nature, but in not less than 7 ears (all AN cases) the onset of deafness was acute

Results of psycho-acoustic differential tests This study confirms the earlier known high sensitivity of the stapedius reflex tests for identifying a retrocochlear hearing dysfunction caused by an expansive process affecting the eighth nerve Also the low reliability of three commonly used psycho-acoustic differential tests is exposed, namely the Fowler test, the speech discrimination test and the tone decay test The scores obtained in these three tests are somewhat surprisingly low in view of the modest requirements here set as criteria for the results to be regard-

[illegible]

[illegible] rejected because of even poorer sensitivity

The unsatisfactory sensitivity of the three psycho-acoustic test used has a tendency to be still more pronounced the better the tone thresholds are (Table VI) As generally seen there exists a relation between tumour size and hearing loss This implies that the common psycho-acoustic tests must be regarded as of only marginal importance for the early diagnosis of acoustic neurinomas From the clinical diagnostic point of view this circumstance is worth consideration as the therapeutic methods of today specifically aim at removal or treatment of small acoustic neurinomas

When discussing the diagnostic value of the psycho-acoustic tests in question, one also has to consider the fact that most clinical audiologists certainly would hesitate to refer a patient for a series of complex and expensive examinations on the basis of a positive outcome of *only one* of the three tests. If we, for example

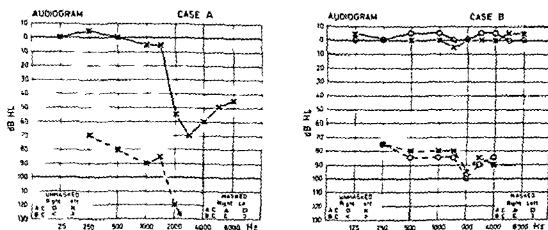


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In this connection it is important to bear in mind that, in cases of expansive processes affecting the eighth nerve, it is quite common to find that no stapedius reflex responses can be elicited by acoustic stimulation of the affected ear, even at maximum stimulus intensities from the audiometer This condition applies, too, in cases with little or no hearing loss and lack of reflex responses should never be disregarded or explained away as caused by, for instance, a subclinical conductive defect Instead the motoric mechanical condition in the opposite recording ear in doubtful cases must be checked by non acoustic stimulation (Klockhoff & Anderson, 1959) The result of this study has clearly demonstrated that with the recording technique used, the absence of stapedius responses can be explained by conductive disorders in only a few per cent of the cases

Of the two criteria used in evaluating the results of the stapedius reflex tests — elevation of reflex threshold and reflex decay, respectively — the reflex decay phenomenon appears to be the earliest sign of dysfunction Of the three cases showing reflex decay as the only abnormality, two had completely normal hearing thresholds To some extent the idea of the reflex decay as the earliest recordable dysfunction is supported by the following curious observation In some of the AN cases treated by stereotactic radiosurgery, the stapedius reflex characteristics recovered during the post-operative period, first as a normalization of the reflex threshold followed by a reduction of the reflex decay, the later in one case leading to completely normal reflex characteristics. (Hirsch et al., 1979) It is tempting to imagine that this recovery process reflects in the reversed order, the course of the reflex abnormalities in the early stages of a process affecting the cochlear nerve

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Specificity of reflex test false positive results The practical value of a clinical diagnostic test is not only determined by its sensitivity to identify a certain pathologic condition but also how specific is the test response to the disorder in question i e, if the proportion of 'false positive' cases is reasonably low In this respect the stapedius reflex tests have demonstrated a high specificity to space-occupying processes involving the eighth nerve every fifth case of pathologically *elevated reflex thresholds* and every third case of patholo[illegible]

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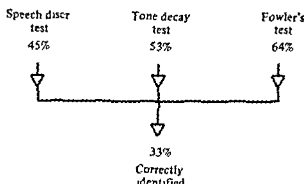


Fig 4 Psycho-acoustic test battery Reduction of correctly identified cases if results of all three tests are required positive

Evaluation of reliability of reflex tests The diagnostic reliability of the reflex tests was checked in the group of cases with the most homogeneous type of lesion, namely the AN group With the technique and criteria used in this study, the *stapedius reflex tests failed in only 2 of the 75 AN cases Calculated in relation to* the total number of patients referred to the Audiological Department on the suspicion of a retrocochlear process during the time period in question (approximately 2000 cases) this gives an incidence of failure in the order of 1 per 1000 cases

To check, as far as possible, that this study gives a true picture of the reliability of the reflex tests in the diagnosis of AN cases certain control measures were performed In Sweden the organization of the medical service in confined local regions offers exceptional possibilities to perform such a control The records of the two neurosurgical clinics serving the Stockholm region were checked against *the records of the Audiological Department and in this way it could be ascertained* that no AN case had been misdiagnosed audiologically and later neurosurgically treated for this disease Further for each new diagnosed AN case the records of the Audiological Department were checked retrospectively to ensure that the patient had not been misdiagnosed at an earlier examination

*Sensitivity of reflex tests In contrast to the low reliability of the psycho-acous*tic tests, the sensitivity of the reflex tests appears to fulfil the high expectations The deceptive audiometric pattern in the form of a unilateral hearing loss with the psycho acoustic differential tests distinctly indicating a cochlear damage but contradicted by positive reflex tests as the only indication of the tumour, was found *in no less than 25 cases If we look at the earliest cases where the hearing thres*holds still were intact the reflex tests proved positive in 27 cases, in 9 of these

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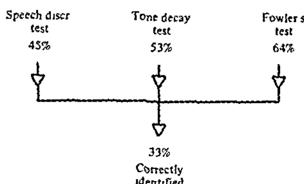


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able reduction in the number of cases submitted to extended neuro-radiologic examination

Comparisons to other studies To make a direct comparison to and between other studies involves many difficulties and in part is simply impossible In Table I (p 4), however, an attempt has been made to survey, as far as possible, the results reported in some representative series of CPA tumours where information about results of one or several of the more common differential tests have been stated

It is to regret that this compilation can only offer a rough outline of the situation Many of the series cover cases with extreme differences in hearing loss ranging from normal hearing thresholds up to hearing impairments where application of audiological differential tests are barely possible Most series lack information on the criteria used for classifying the outcome of the individual tests as positive or negative, here the authors' statement must be accepted blindly Some results are presented in a way that renders a correct interpretation impossible So for instance, the results of the commonly used speech discrimination test often are given as bulk figures, such as percentage of all cases showing reduced discrimination This mode of presentation of results has not been regarded suitable and these are consequently excluded in the compilation, a circumstance that explains the low number of entries in the speech discrimination column of Table I It is the authors' opinion, that any estimation of a patient's discrimination ability for each individual case must be related to tone threshold elevation and configuration Further, differences in test technique, not only in stapedius reflex examinations (Hirsch & Anderson, 1980) but also in the psycho-acoustic test procedures can be expected to contribute to differences in results

In spite of all these factors of uncertainty hampering the compilation of Table I, the general tendency is readily distinguished The results of the stapedius reflex tests and reflex decay tests show with few exceptions markedly higher scores than any of the psycho-acoustic tests In quite a number of the studies, the results of reflex tests approach or even reach the remarkably high accuracy of 100% These observations are supported and even emphazised by the results of the present study where the superiority of the reflex threshold test and the reflex decay test in the early audiological indentification of CPA cases is demonstrated

Recruitment versus reflex threshold level As pointed out in the introduction, many authors have treated the stapedius reflex threshold test as a true test for the psycho-acoustic phenomenon of loudness recruitment (Metz, 1952, Thomsen & Terkildsen 1975) Some have even gone so far as to describe stapedius reflex activity as 'loudness governed' (Djupesland et al , 1976) Although such conceptions, in view that all knowledge concerning the physiological properties of the stapedius reflex must be regarded as both deceptive and irrational, it does not entirely rule out the possible existence of some sort of parallelism between the ear's stapedius reflex threshold and the ear's loudness function Such a parallelism seems to prevail in ears with hearing threshold impairment and *normal* reflex threshold level, the loudness sensation evoked in such ears when stimulating at reflex threshold level intensity, equals closely the loudness sensation evoked in a normal ear when stimulated by same intensity This parallelism with respect to, on the one hand

the reduced span between impaired hearing threshold and the normal reflex thres hold and on the other hand the reduced span between hearing threshold and the 85 dB loudness level (equal to the HL at which reflex threshold normally is reached) forms the basis for the conception 'Metz recruitment test'

That no such general parallelism can be expected in ears with pathologically elevated reflex thresholds has been pointed out in earlier papers (Anderson et al, 1970, Hirsch & Anderson 1980) This observation is confirmed by the present study where it is demonstrated that in ears with reflex threshold elevation caused by CPA tumours the loudness function may be found normal and the Fowler test indicating considerable recruitment (Fig 5)

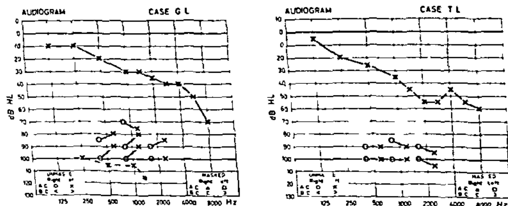


Fig 5 Audiograms illustrating that elevation of stapedius reflex threshold very well can appear in ears with recruitment as measured by the Fowler test
Case G L Man 33 years acoustic neurinoma on left side
Case T L Man 20 years, acoustic neurinoma on left side
In case T L reflex threshold was elevated above limit of audiometer output In both cases hearing and reflex thresholds were within normal limits on opposite ear

The conclusion is, that the 'Metz recruitment test' undoubtly shows a close agreement with the true psycho-acoustic recruitment tests under the condition that the ear under test has a normal reflex threshold but that the reflex threshold test is not reliable for determining the presence or absence of recruitment in ears with elevated reflex threshold This study also emphasizes the danger of basing the evaluation of a possible absence of recruitment by requiring a parallel eleva tion of hearing threshold and reflex threshold Instead, the two thresholds should always be treated as separate functions each bearing its own diagnostic informa tion

Results of some non-audiologic tests As mentioned in the foregoing only a few of the non acoustic tests have been performed systematically during the years and uniformly enough to permit a reasonably reliable evaluation of results With re spect to the results of the ENG examination only about half of the AN cases demonstrated the classic alarming signal totally extinguished caloric responses

Although direct comparisons to other studies not are possible due to differences in technique and criteria the incidence in the present study seems rather low, a circumstance that probably can be ascribed to the fact that the case material presented here contains far less advanced AN cases

Among non acoustic methods, radiography has undoubtly been subject to the most extensive development during the years covered by this study However, as the technique for tomography of the internal acoustic meatus has remained fairly unchanged, the results from these examinations were compiled for analysis The proportion of positive findings in the form of enlarged or destructed porus amounts in the AN cases to 68% When considering that this result was obtained in a selected material of 'early' AN tumours, it undoubtly must be regarded as fairly satisfactory

Age distribution As shown by Fig 2, (p 11), the age distribution curve of the AN cases in the present material reaches a maximum in the age group 56–60 years and then rapidly falls off towards higher ages The same tendency is found in larger series (Johnson 1977) As it is a well known fact that the total occurrence of sensorineural hearing disorders increases heavily above 50 years of age, it seems rather surprising that the number of AN cases here should decrease As the AN cases in the present study were extracted from an ordinary audiological/clinical material with considerably higher mean age, this finding cannot be explained as caused by a selection of the material according to age The only conclusion that may be drawn from this observation is that the probability of incurring an AN with its clinical manifestations seem to be reduced above middle age

Conclusions

The results of this study confirm the indisputable value of the stapedius reflex tests in the audiological diagnosis of tumours affecting the eighth nerve The reflex tests when performed carefully and with appropriate technical equipment, are sensitive enough for an early detection of these processes, a demand arisen as a consequence of new methods of treatment

The study also demonstrates that the initial subjective symptoms as well as audiometric pattern and outcome of common psycho-acoustic differential tests, show considerable individual variations and frequently are directly misleading The experience based on more than 20 years of stapedius reflex measurements in clinical practice stresses the opinion that all cases where the slightest suspicion of a tumour is present even if the hearing threshold is normal, should be submitted to stapedius reflex examination

SUMMARY

This study concerns the audiological findings in 96 cases of tumours affecting the eighth nerve, and with a maximum tone threshold loss of 60 dB on the affected side Age ranged from 5 to 81 years with a mean age of 43 years. 44 patients were women, 52 men Seventy-five tumours were classified as acoustic neurinomas (in one case bilaterally) and the main part of the remaining 22 tumours was diagnosed as meningeomas cholesteatomas and gliomas. Of the acoustic neurinomas, 38 measured 2 cm or less in diameter whereas the other tumours in general were reported to be of larger size

The mean HL for the total material was 32 dB, not less than 28 ears showed tone thresholds within normal limits age and sex taken into account In the psycho-acoustic tests, the proportion of results indicative of a retrocochlear lesion was found to be about 50% (speech discrimination test = 45%, tone threshold decay test = 53%, absence of recruitment in Fowler's test = 64%), only one-third of the cases showed pathological results in all three tests With the material grouped in acoustic neurinoma cases and other cerebello-pontine angle tumours, respectively, it was demonstrated that the latter group contained a higher proportion of ears with normal hearing thresholds and a smaller proportion of pathologic results in the psycho-acoustic tests

An analysis confined to the results in the acoustic neurinoma cases showed that the sensitivity of the common psycho-acoustic tests decreased the less pronounced the hearing threshold impairment The conclusion is that the psycho-acoustic tests have a poor ability to identify retrocochlear lesions caused by tumours affecting the eighth nerve, and that these tests cannot be regarded as reasonable reliable for the identification of such processes at an early stage

With the stapedius reflex tests (reflex threshold test and/or reflex decay test), pathologic results were recorded in 98% of the ears affected by a tumour In 60% of the ears the stapedius reflex thresholds were elevated above limit of audiometer (120 dB HL), in another 35% the reflex threshold was recorded at abnormally high level The reflex decay test could be performed in 36 ears and in 23 ears a clearly pathologic reflex decay was recorded, further, 9 ears showed some reflex decay but not to an extent that fulfilled the current criterion for pathology In 4 ears no indication of reflex decay was seen in the recordings

In two cases as well stapedius reflex tests as the psycho-acoustic differential tests failed to identify the retrocochlear disorder Both cases belonged to the acoustic neurinoma group

The experience gained from this study emphasizes the value of a carefully performed stapedius reflex examination in the efforts towards an early audiological diagnosis of tumours affecting the eighth nerve All cases where such a process is suspected even if the hearing threshold is completely normal, should be subjected to stapedius reflex examination

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Conclusions

The results of this study confirm the indisputable value of the stapedius reflex tests in the audiological diagnosis of tumours affecting the eighth nerve The reflex tests, when performed carefully and with appropriate technical equipment, are sensitive enough for an early detection of these processes, a demand arisen as a consequence of new methods of treatment

The study also demonstrates that the initial subjective symptoms as well as audiometric pattern and outcome of common psycho-acoustic differential tests, show considerable individual variations and frequently are directly misleading The experience, based on more than 20 years of stapedius reflex measurements in clinical practice, stresses the opinion that all cases where the slightest suspicion of a tumour is present even if the hearing threshold is normal, should be submitted to stapedius reflex examination

SUMMARY

This study concerns the audiological findings in 96 cases of tumours affecting the eighth nerve, and with a maximum tone threshold loss of 60 dB on the affected side. Age ranged from 5 to 81 years with a mean age of 43 years. 44 patients were women, 52 men. Seventy five tumours were classified as acoustic neurinomas (in one case bilaterally) and the main part of the remaining 22 tumours was diagnosed as meningeomas, cholesteatomas and gliomas. Of the acoustic neurinomas, 38 measured 2 cm or less in diameter whereas the other tumours in general were reported to be of larger size.

The mean HL for the total material was 32 dB, not less than 28 ears showed tone thresholds within normal limits age and sex taken into account. In the psycho-acoustic tests, the proportion of results indicative of a retrocochlear lesion was found to be about 50% (speech discrimination test = 45%, tone threshold decay test = 53%, absence of recruitment in Fowler's test = 64%), only one-third of the cases showed pathological results in all three tests. With the material grouped in acoustic neurinoma cases and other cerebello-pontine angle tumours, respectively, it was demonstrated that the latter group contained a higher proportion of ears with normal hearing thresholds and a smaller proportion of pathologic results in the psycho-acoustic tests.

An analysis confined to the results in the acoustic neurinoma cases, showed that the sensitivity of the common psycho-acoustic tests decreased the less pronounced the hearing threshold impairment. The conclusion is that the psycho-acoustic tests have a poor ability to identify retrocochlear lesions caused by tumours affecting the eighth nerve and that these tests cannot be regarded as reasonable reliable for the identification of such processes at an early stage.

With the stapedius reflex tests (reflex threshold test and/or reflex decay test), pathologic results were recorded in 98% of the ears affected by a tumour. In 60% [illegible]

pathologic reflex decay was recorded, further, 9 ears showed some reflex decay but not to an extent that fulfilled the current criterion for pathology. In 4 ears no indication of reflex decay was seen in the recordings.

In two cases as well stapedius reflex tests as the psycho-acoustic differential tests failed to identify the retrocochlear disorder. Both cases belonged to the acoustic neurinoma group.

The experience gained from this study emphasizes the value of a carefully performed stapedius reflex examination in the efforts towards an early audiological diagnosis of tumours affecting the eighth nerve. All cases where such a process is suspected even if the hearing threshold is completely normal should be subjected to stapedius reflex examination.

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With the stapedius reflex tests (reflex threshold test and/or reflex decay test), pathologic results were recorded in 98% of the ears affected by a tumour. In 60% of the ears the stapedius reflex thresholds were elevated above limit of audiometer (120 dB HL), in another 35% the reflex threshold was recorded at abnormally high level. The reflex decay test could be performed in 36 ears and in 23 ears a clearly pathologic reflex decay was recorded, further 9 ears showed some reflex decay but not to an extent that fulfilled the current criterion for pathology. In 4 ears no indication of reflex decay was seen in the recordings.

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